SYLLABUS OUTLINE FOR GENETICS LECTURE AND LABORATORY

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This work is intended to be used as a teaching tool in conjunction with the text cited. It is written in outline format, highlighting the major concepts of each pertinent chapter. In this format, the concepts can be expanded upon at the discretion of the instructor. This work is to be used as a guide for lecture. The basic concepts contained in the outline are in such a format as to be able to work in more information regarding the subject matter if needed. The instructor can work from this outline as a starting point. Major topics in the chapters are highlighted, making lecture notes for the instructor easier to do.

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INTRODUCTION

My life was simple while I was earning my bachelor's degree in Biology. I had developed a love of Science from the example my father set for me. He would read magazines, watch television programs, and research new technology just to gain more knowledge in the field of science. He introduced me to every aspect of science. I wanted to understand why he found these ideas and concepts fascinating. Seeing my father engulf any knowledge he could in the field made me curious. The passion and enthusiasm he had in sharing what he knew with me was infectious. I wanted his passion and determination. I wanted to know what he knew. Through that curiosity came a love of my own. As I graduated from High School and entered college, there was no doubt in my mind that I would get my degree in science. I earned my bachelor's degree in biology. During my junior year, when all my basics were finished, I took a number of diverse biology classes. I did this to discover what area of science meant the most to me. Everything from ornithology to human anatomy to genetics is now forever listed on my transcript. I was using this time to decide exactly what I was going to do with my degree, which direction I would take and what career path I was to go into. To get the finishing credits I needed to graduate, I was a teaching assistant for Animal Life laboratory and Basic Biology laboratory. At first it was a requirement, after a few weeks, it was a desire. I enjoyed being in front of that class sharing knowledge with future biology majors. The epiphany came while I was making notes for my lab. I realized that I could go to medical

school and practice medicine. I could do research for a pharmaceutical company. I could finish at Texas A&M University and become a veterinarian. I also realized that I would be adequate at these tasks. I would be mediocre, skilled enough to get by, but not to be a pioneer in the field. It troubled me why I felt this way. I thought about it for a while and realized there was a new purpose in my life. God granted me the gift of learning. I love to learn and I will never stop. The other gift he gave me was the ability to share. My epiphany was clear: I love to share what I absorb- I love to teach! Whereas I would be a capable physician, researcher, or veterinarian surgeon, I believe that I would be a better professor. A calming sense of peace came over me as I understood this concept: God did not place me on this Earth and give me all he has given me so I can find the cure for cancer. He placed me here to teach the person that might.

During my Masters degree I had the opportunity to teach laboratories. That experience solidified my career decision. I learned and returned knowledge. The most important aspect of teaching that I came away with was that the students have just as much to teach as they have to learn. I was excited when the students grasped the concepts I was trying to teach. I was pleased when they asked questions that showed they were tying in what I discussed with the knowledge they already possessed. I believe that teaching is a two way street. The person teaching passes out the knowledge and it is up to the student to use that knowledge in new and creative ways. It need not be dry and rudimentary, yet full of possible creative outlooks and new ways to improve and advance. When I was growing up, I was taught learning was not just about memorization, but applying the information in new ways to expand my mind. I also believe that teaching will be an arena where I will personally continue to learn. The feedback from the students

helps me to explore other avenues in science I might not have thought about without their input. I truly believe if a person does not continue to learn throughout life there will be an empty place within. The teaching profession is an area where I will be in a constant state of learning. It is a wonderful benefit to an already wonderful career. It may sound presumptuous of me to say I might teach the person who will find the cure for cancer. Even if this person does not pass through my class, I hope that I will be able to spark some interest in others. My main goal in life is to touch a life and introduce others to science. If I can teach the basics to a person who will in turn use that knowledge to enrich their lives or the lives of others, then my job is done. Science is a building block. Concepts build on top of each other and keep building with more interest and research. If I can teach my lesson plans while at the same time exhibit the passion I have for this subject, I may be able to peak the curiosity of a student like my father did in me. To have someone leave my lecture and ask themselves why I love this subject so much and be curious enough to explore the question on their own would be a reward. Every person that passes through my classroom has the ability to change the world, themselves, and me at the same time. I look forward to teaching and being taught. I strive to learn as much as I can so I in turn will have more to share.

This syllabus project gave me insight on teaching. Taking a concept and making it understandable is not as easy as it sounds. This project has helped me look at ideas differently. To teach is not just the ability to stand in front of a group and speak. The teacher must be patient, tolerant, and understanding. It is one thing if I understand the concept placed before me; it is quite another to explain the concept. I have just begun to learn different ways of explaining an idea. To make every aspect clear and concise and

demonstrating how it works within the subject of the lecture. Since beginning my masters' degree, I have learned that I still have a lot to learn. I am dedicated to that challenge and strive to improve every day. I learn to be a better teacher with every lab I teach. I learn to be a better student with every class I take. This project has taught me not only what I have put in this paper, but what I still have left to learn. Becoming a professor is not an easy task, but nothing worth having ever is. I am thankful I chose this project for what it has taught me, and what is has shown me that I need to work on and improve. I am a visual learner, and throughout this thesis I imagined outlines, graphs, and transparencies I can use to display the knowledge I am trying to convey. For most people, if they read the information, listen to the information, and have a visual reference to associate with the information, it will be ingrained in their memory. As I read through the textbook and wrote down my lecture guideline, my brain was filled with pictures of DNA structure, antibodies, phenotypic results of teratogens, and the process of gametagenesis. Some concepts are easier grasped when combined with a diagram of the specific structure. Since this course outline is designed for upper level science majors, it is important for the students to know how to distinguish structures in a diagram, to draw specifics, and to understand how a process works in picture form. There are aspects of science that are entirely visual. Place a scientist in a lab with a microscope armed only with the technical knowledge of what he is looking for and he will be lost. The ability to draw and to recognize is essential for a perspective science major. As a teacher, I hope to instill this characteristic in my students.

Genetics is a newly found passion of mine. It is a relatively new subset of science that is rapidly changing. My bachelor's background mainly focused on mammalogy and

human anatomy. I have taken great pleasure in expanding my knowledge in the field of genetics. I feel with as new as a discipline as molecular genetics is, future students will focus their studies more in this field. I want to be ready for the influx of eager minds yearning to practice in this field. The future of genetics is almost limitless. I intend to keep up with any changes or new advances, and possibly create some of my own in order to pass on the knowledge to my students. I feel strongly that teaching genetics can not be responsibly done without focusing on ethics. This can be a difficult task with the many religious beliefs that will flow in and out of a classroom. Taking a cue from genetic counseling, an "in-directive" approach may help in this situation. All the issues and their consequences, repercussions, and morality needs to be discussed within the lectures. To appreciate and respect every individual religious and moral belief is key. An in-directive approach will inform without discounting a personal belief. These discussions should bring about the opportunity for students to learn and understand beliefs that are foreign to them. Expanded knowledge in the morals of others will enlighten others of the world around them. The blinders will be removed and opinions can be heard. I feel this is a valuable life lesson, in addition to an important part of genetics. Beyond the classroom, a teacher does not have control on how the knowledge is used. The study of genetics has vast possibilities and even larger ethical issues. I hope to convey the information needed for students to make responsible moral judgments when they leave school and are using genetics in the world. Students need to understand the moral responsibility attached to research and improvements in this field. The invention or discovery of a life altering procedure could carry catastrophic repercussions if mishandled. It is hard to define how far is too far in the way of technology. To expose students to various moral and religious

beliefs will hopefully instill a sense of sympathy and respect to certain taboo boundaries they may encounter. Genetics should take a cue from the physicist Niels Bohr who worked on nuclear fission, energy and the atom bomb. While in the middle of his research he realized the awesome power nuclear energy was capable of, and wrote letters to Winston Churchill and President Roosevelt urging them to develop international cooperation regarding nuclear energy. Advancements in the field of genetics have the capability of possessing that kind of power, and should be approached cautiously and conscientiously. I want the students to learn what I teach, but also to think for themselves.

I have geared this class toward junior and senior college level biology majors with interests in the medical field or genetics. The text was chosen due to its content of genetics from the cellular level. With chapters that encompass the chemistry of the gene, genetic linkage, chromosomes and heredity, intense study of the function of DNA, and application of genetics, I feel that this is a wonderful text for a cellular look at genetics. A prerequisite of cellular biology would be helpful. I have tried to take the knowledge a student would already have in this area and expand upon it genetically. The course is more structure and function than counseling or specific diseases or anomalies. A good base rooted knowledge in exactly how DNA, genes, and chromosomes replicate and synthesize should make any further learning in genetics easier. Learning to identify the correct forms of genes and chromosomes and comparing these to disorders that posses incorrect forms is a great ground level understanding of genetics. The students should not only to be able to identify a deletion or translocation, but explain how it came to be. What happened at the cellular level for this to occur? The main objective of this course is to arm the student with the knowledge to answer such questions. Whether this knowledge is

used in a laboratory, a classroom, an operating room, or to settle a bet at a cocktail party, I feel that the study of genetics can enrich a life. I have written this syllabus per instructions in an outline format. I have outlined the pertinent chapters into sections of importance. The information contained in each section is the basic concept needed to be emphasized during lecture. Since this paper is in an outline format, it is easy to expand upon. The basic concepts are contained, and are in such a form as to be able to work in more information regarding the subject matter if needed during any lecture. The main function of this design is the ability of any instructor to be able to use this outline in conjunction with the book to teach a college genetics course. The laboratory section is very flexible. Certain materials may not be available, so different options are noted. I feel strongly that a *drosophila* lab is essential in a genetics lab. They are relatively easy to work with, inexpensive and have great variability in offspring. I feel one of the most important aspects of lab is learning to use a plethora of equipment. It is important for students to obtain accurate results, for their self esteem if nothing else. Yet, lab is a wonderful opportunity to be exposed to different types of equipment and techniques and learn how to use them. The lab not only is a tool to introduce equipment to the students, but to reiterate the knowledge obtained during lecture. Some of the labs are hands-on wet labs, while others are more paper oriented to put to use concepts learned in lecture. If this lab outline were to be used, the stock of the lab and availability of equipment would need to be taken under consideration. Some of the labs outlined will work, others may need to be augmented, while other labs may need to be added to fill the requirement.

OUTLINE

Genetics (Chapter One)

I. What is Genetics?

A. Definition- termed by biologist William Bateson; Gene termed by Johansson.

B. Consists of three branches which reinforce each other:

1. Transmission genetics – the study of the transmission of traits from one generation to the next.

2. Molecular genetics – the study of the structure and expression of genes at the molecular level.

3. Population genetics – the study if the variation of genes between and within populations.

C. Careers in Genetics

1. Genetic Counselor; calculates the risk of recurrence of inherited disorders in families. Counsels patients after diagnosis, help with treatment planning, testing. Indirective counseling- give options but do not give advice on what course of action to take. *Pamphlets, etc to help explain.

2. Researcher- medical advances, identifying gene causes of illnesses, awareness, human genome project-2005; 50,000-100,000 genes.

3. Teacher- etc.

D. Use of Genetics

1. Abnormal to explain the normal; yet not able to predict severity.

2. Prenatal testing-know disorders before birth, risk calculations. *Briefly touch on CVS, amniocentesis, sonogram, triple screen, etc.

3. Crime Lab- DNA testing for identification, fingerprint I.D. kids

4. Why study? Ancient civilizations used genetics to raise better crops and breed healthier animals. Cloned genes are used for insulin for diabetics and interleukins for cancer, etc.

II. Understanding Genetics means understanding basic principles;

A. Review of Cells- (Make sure cell structure and function is clearly understood)

1. Prokaryotic Cells

a) Include bacteria and cyanobacteria (Eubacteria)

b) Simple- lacks a nucleus

c) Organized and efficient

d) Nucleoid-contains a single circular DNA molecule

e) Contains- nucleoid, ribosomes, fats, protein, carbohydrates, pigments, cell membrane, cell wall, plasma membrane, and single chromosomes.

2. Eukaryotic cells

a) 100x volume of prokaryotic cell

b) Organized by organelles- “bags within a bag”

c) Nucleus enclosed in a nuclear envelope, within a nucleolus, ribosomes formed.

d) Contains-endoplasmic reticulum, vesicles, Golgi body, mitochondria, lysosomes, peroxisomes.

e) Nucleus – discrete structure within a cell that contains genetic material called DNA.

f) Chromatin – Network of DNA fibers. During mitosis the DNA molecules coil around specialized proteins to form the chromosome structure. DNA combined with five basic proteins called histones and a large group of nonhistone proteins.

g) Chromatin exists as heterochromatin, which is condensed and transcriptionally inactive; and euchromatin, which is extended and at least potentially active.

h) The chromatin is organized by folding;

(1) First order is represented by a string of nucleosomes. It contains four pairs of histones (H2A, H2B, H3, H4) in a ball which are wrapped about 200 base pairs of DNA. The fifth histone, (H1) binds to DNA outside the ball.

(2) Second order – involves a coiling of the string of nucleosomes into a 25 nm thick fiber called a solenoid. This folding is mediated by H1-H1 interactions.

(3) Third order – involves looping of the 25 nm fiber into a brushlike structure with the loops anchored to a central matrix.

i) Centriole – Cylindrical structure composed of microtubules. Paired centrioles organize the formation of spindle fibers during mitosis.

j) Endoplasmic Reticulum ER– Network of membrane throughout the cell. Rough ER is studded with ribosomes. Bound ribosomes of the ER synthesize proteins that are destined for secretion or are to be incorporated into the membrane or specific vacuoles.

k) Golgi Body – Continuation of membrane network of the ER.

l) Ribosomes – Free ribosomes in the cytosol synthesize proteins that remain in the cell and are not transported through the ER and Golgi body.

m) Mitochondrion – Site of energy production in the cell. Consists of double membrane system. Outer is smooth, inner has convoluted folds. Can divide independently of the cell and contains its own circular double stranded DNA.

n) Eukaryotic DNA combines with basic protein molecules called histones to form structures known as nucleosomes. Histones – H2A, H2B, H3 and H4, form a ball that is wrapped around a stretch of 200 base pairs of DNA. Histone HI bonds to DNA outside this ball.

o) Nucleosome – important functions of the nucleosome. Universality of the nucleosome and extreme evolutionary

conservation. The difference between H4 in cows and pea plants is only two amino acids out of 102. Nucleosome provides the first order of condensing or coiling of the chromosome fiber.

p) Contain three RNA polymerases: RNA I, RNAII, RNA III.

q) Many eukaryotic genes contain or reside close to enhancers. They are not promoter elements, but they greatly stimulate transcription of nearby genes. Some reside near silencers that inhibit transcription. Enhancers and silencers interact with different transcription factors that control promoter activity.

III. Cell Composition

A. Macromolecules- carbohydrates, (sugars and starches), lipids, (fats and oils), proteins, and nucleic acids, etc.

B. Discuss and review composition of cell.

IV. Cell Cycle

A. Mitosis – nuclear division that results in the production of two identical daughter nuclei.

1. Interphase - preparation for division.

a) Two Gap Phases (growth) and one synthesis phase. Total time 24 hours. G1=9-11 hours, S=5-9 hours, G2=3-4 hours. Mitosis=1 hour.

b) Cannot count network of chromosomes, DNA replicated.

2. Prophase- DNA coils tightly, visible, microtubules.

3. Metaphase- Chromatids align.

4. Anaphase- cell indents, chromosomes separate.

5. Telophase- (Interphase II) two cells, membrane reappears.

(Show slides of mitosis)

This discussion correlates with Lab II – Mitosis

B. Meiosis

1. Early prophase- replicated chromosomes condense and become visible as a tangled mass within the nucleus

a) Prophase I can be broken down into five different parts.

(1) Leptotene – (thin thread stage) is marked by the appearance of the chromosomes as long threads when seen through a light microscope.

(2) Zygotene – (joined thread stage) homologous chromosomes pair side by side and gene by gene with each other. Synapsis occurs during zygotene and is a key difference between meiosis and mitosis. When the two homologous chromosomes consisting of four chromatids are paired the structure is bivalent.

(3) Pachytene – (thick thread stage) consists of a shortening and thickening of the bivalent and is the stage during which synapsis is complete. During this stage portions of the homologous chromosomes may be exchanged called crossing over or recombination. The structure that allows crossing over is the synaptonemal complex which is made

of protein and DNA that is found between the synapsed homologues.

(4) Diplotene – (double thread stage) the homologues begin to separate as the synaptonemal complex breaks down, particularly in the region on either side of the centromere. The sister chromatids remain attached at the centromeric region. The homologous chromosomes generally have one or two areas called chiasma. Chiasma (chiasmata – plural) is the physical evidence that recombination occurred earlier when the homologues were synapsed.

(5) Diakinesis – characterized by shortened chromosomes and the terminalization of the chiasmata. The chiasmata appear to move to the ends of the chromosomes.

b) Late prophase- pairs align and the homologs cross over

c) Metaphase- spindle fibers align the homologs

d) Anaphase-the homologs move to opposite poles

e) Telophase- the genetic material is partitioned into two progeny nuclei, each containing only one homolog from each pair.

2. Second phase of meiosis- similar to mitosis

a) Prophase II- the chromosomes are visible

b) Metaphase II- the spindle fibers align the chromosomes

c) Anaphase II- centromeres part, each chromatid pair divides into two chromosomes, which move toward opposite poles.

d) Telophase II- two separated sets of chromosomes are enclosed in separate nuclei, and then partitioned into two progeny cells.

e) Yield = four haploid progeny cells. Eight strands of DNA- two successful divisions gives four strands- one set of double stranded DNA in each 23 chromosomes in humans.

This discussion correlates with Lab III – Meiosis

V. Spermatogenesis-

A. Process begins with a diploid cell called a spermatogonium, which divides into two daughter cells.

B. One of these cells turns into a mature sperm while the other remains a stem cell which retains the ability to divide again.

C. Several spermatogonium are joined by cytoplasm and continue meiosis, replicating their DNA and become primary spermatocytes.

D. In meiosis I each spermatocyte divides into two equal haploid cells called secondary spermatocytes, then go through meiosis II and divide into two more equal cells called spermatids.

E. These spermatids develop the flagellum that propel the sperm. 0.0023 inch long and travel around seven inches to get to the ovum.

F. Tail base is made of mitochondria and ATP molecules to give energy for transport

G. The front end of the sperm, the acrosome, contains enzymes that help the sperm to penetrate the oocyte.

H. Acrosome contains DNA wrapped around proteins.

I. Protection against chromosomal anomoly- damaged sperm will not specialize, can not swim.

J. Extra chromosome makes sperm too heavy to swim.

K. Risk of an older male passing along a birth defect is low; male always making new sperm. A primary spermatocyte will change to a sperm in 60 days.

(Show slide of spermatogenesis)

VI. Oogenesis-

A. Process begins with a diploid cell, an oogonium.

B. The oogonium will accumulate cytoplasm and replicate its DNA becoming a primary oocyte.

C. The division of these cells will produce daughter cells of varying sizes.

D. During meiosis I the primary oocyte will divide into two haploid cells.

E. One of these cells is larger, the secondary oocyte and the other smaller cell is the polar body.

F. The polar body may continue to divide into another polar body and an ovum, a mature egg that contains cytoplasm.

G. Polar body may continue to divide or be absorbed by the body.

H. Polar bodies can be used for genetic testing. Polar body contains half the chromosomes, the egg gets the other half.

I. At birth a human female has 1-2 million oocytes in prophase I.

J. During puberty, Meiosis I continues in one or several oocytes each month but stops at metaphase II.

K. Hormones release one 2' oocyte from an ovary- ovulation.

L. Meiosis in females is complete only if there is fertilization.

M. 400 oocytes will be used from puberty to menopause.

N. One in three oocytes and sperm will meet and grow, divide and specialize into a fetus.

(show slide of oogenesis)

VII. Development: *Brief overview

A. Within 30 hours of the egg and sperm meeting it is ready for the first mitotic division. Totipotent = each cell makes a total fetus. For in vitro fertilization, can take one cell out of the 8 cell phase to test for genetic disorder. If negative, can transfer zygote to uterus.

B. Fertilization occurs in the Fallopian tubes.

C. The zona pellucida (covering of the egg for protection that the sperm goes through) will disappear at day five after fertilization.

D. Day six, implantation occurs on the wall of the uterus and the cells erode away into the endometrium.

E. Trilaminar – embryo develops into three layers.

F. Four weeks after conception – development of the heart and spine are susceptible to birth defects. Prime time for miscarriage; 25% of pregnancies end in miscarriage during this time.

G. During week 9 marks the beginning of the fetal period, no longer considered an embryo. This time is important for growth and brain development. Grows about one pound per week.

VIII. Problems:

A. Non-disjunction = cell does not separate properly. Some cells have more chromosomes than others, happens in the egg.

B. Cell division is a controlled, precise process. If the process is sped up, could cause a genetic disorder. Progeria = premature aging disorder, i.e. Werner syndrome.

C. Hayflick limit = the number of cell divisions that a cultured cell will undergo before dying. @ 50 generations typical for human cells. Cancer cells can repair telomeres after replication, they can be immortal, normal cells can not. Cancer cells are capable of defying the Hayflick limit because they do not degrade their chromosomes. Possible cancer treatment – turn off telomeres in cancer cells.

IX. DNA structure and function-

A. DNA is a group of polymers whose job is to carry information that will make proteins. Function = replication, storage of information, variation by mutation. Faithfully replicates itself and orchestrates synthesis of all proteins in the cell.

1. Nucleic acids- are formed from monomers called nucleotides consisting of a simple sugar, (deoxyribose), a phosphate group, and a nitrogenous base.

2. Four nitrogenous bases = Adenine, Cytosine, Guanine, Thymine. Adenine and Guanine are purines. Cytosine, Thymine, and Uracil are pyrimidines.

3. Complementary base pairing means certain bases can only be paired with a specific match. Adenine = Thymine (Uracil in RNA) Cytosine = Guanine

4. Allows for hydrogen bonding which allows the two polynucleotide chains that are the backbone to stay together.

5. Allows for small to large base pairing to equate sizing of molecules.

6. Hydrogen bonding gives the twist to the helix, prevents distortion, and gives and accurate copy of DNA.

7. Helix turns right, anti-parallel, one turn = 34 A or 3.4 nm = 10 bases. 20 A or 2.0 nm in diameter. (A= angstroms)

8. DNA is wrapped around eight histone proteins, which are arginine and lysine rich (amino acids) to form nucleosomes. Octamer of histones is two of each: H2A, H2B, H3, and H4.

X. Replication and Synthesis

A. Semi-conservative replication – One strand of the parental double helix is retained in each of the daughter strands.

B. DNA duplex is undone by breaking hydrogen bonds holding the base pairs together, forming a "Y" shaped molecule called a replication fork.

C. DNA polymerases move down the then single stranded arms in a 5' to 3' direction. The strands are anti-parallel, one runs 3' to 5', the other 5' to 3'. Synthesis done in only the 5' to3' direction.

D. New nucleotides are only added to the 3' end of the chain. Parent strand runs 3' to 5', and daughter strand (leading strand) runs 5' to 3'.

E. The new fragments on the daughter strand can be 100 – 200 nucleotides in length and is known as the lagging strand.

F. The fragments made are called Okazaki fragments.

G. Replication is a semi-conservative process, meaning when each strand separates each strand can be used as a template. Replicated DNA is known as a replicon. Chromosomes of plasmids, viruses, and bacteria have one replicon. Initiated by DNA polymerase at 1600 nucleosides/ second.

H. Replication is part of the cell cycle and occurs during the S Phase

I. Replication is usually bi-directional. It starts at a fixed point- origin of replication – and two replication forks move in different directions away from the starting point.

J. Replication in prokaryotes and eukaryotes is semi-discontinuous; leading strand grows continuously, 5' to 3' in the direction of motion of the replication fork. The other strand (lagging) grows discontinuously in pieces (Okazaki) that are made 5' to 3' in the opposite direction of the replication fork.

K. Protein – The genetic allows the reading of the sequence of bases in a DNA strand. To make a protein, an enzyme makes a copy of one of the DNA strands (it is actually RNA). The RNA (mRNA) carries the instructions to the cell's protein factories called ribosomes. They "read" the genetic code and put together a protein according to its instructions.

L. DNA polymerase needs a primer, usually a small piece of RNA to initiate DNA synthesis. As the helicase unwinds, it introduces superhelical strain into the DNA. DNA gyrase releases the strain and allows replication to continue.

M. Genetic code – Khorana and Nirenberg cracked the code in 1961. A codon is a three base code word that stands for one amino acid. Out of 64 possible three base codons, 61 are for amino acids and three are stop codes that release the protein.

N. DNA and genes – Genes are made of DNA, some viruses are genes of RNA. The melting temperature , Tm, of DNA is when two strands denature. Genes participate in 1) replication, 2) to hold information, 3) accumulate change. Gene information is expressed by 1) transcription, synthesis of messenger RNA, 2) translation, synthesis of proteins using instructions found in messenger RNA.

XI. Priming DNA for synthesis:

A. RNA polymerase begins synthesis or transcription, DNA does not have this. The missing piece in DNA is a primer or starter piece of nucleic acid that the polymerase can grab onto and extend by adding nucleotides to its 3' end. The primer is actually a short piece of RNA.

B. If DNA is unidirectional, only one fork is active and DNA replication proceeds away from the other stationary fork. If replication is bi-directional both forks are active and replication proceeds in both directions away from a common starting point. Bi-directional is more common.

C. After DNA polymerase has completed its function, all the nucleotides have been replaced expect for a nick, a single stranded break. A nick translation is the movement of a nick to a new location. DNA ligase is used to fill in the nick making a new phosphodiester bond to seal the DNA.

XII. Transcription-

A. The transfer of genetic information from DNA by the synthesis of an RNA molecule copied from DNA template.

B. This results in the process of the three types of RNA, messenger RNA, transfer RNA, and ribosomal RNA.

C. rRNA forms a ribosome, mRNA carries the message specific for a protein, tRNA transfers amino acids during translation.

D. Transcription is catalyzed by RNA polymerase.

E. Three phases of transcription –

1. Initiation – the enzyme recognizes a region called the promoter which lies up from the gene. The polymerase binds tightly with the promoter and causes melting or separation of the DNA. 12 base pairs are melted. The polymerase then starts building the RNA chain. The building blocks are ribonucleoside triphosphates: ATP, GTP, CTP, and UTP. The initiating substrate is a purine nucleotide. The other blocks are added to form the initial phosphodiester bond and initiation is complete.

2. Elongation – RNA polymerase directs the sequential binding of ribonucleotides to the growing RNA chain in the 5' end. As this occurs, it moves along the DNA template and the melted DNA moves with it. The melted region exposes the bases of the template one by one so they can pair with bases. Once the transcription machinery passes the two strands wind around each other to re-form the double helix.

3. Termination – terminators signal the end of work. Work with RNA polymerase or another protein to beak the connection of the RNA and the DNA template.

XIII. Translation –

A. Most genes contain the information for making one polypeptide, polymer of amino acids linked together through peptide bonds. Ribosomes are the cells primary protein factories.

B. Ribosomal subunits can be dissociated into their component parts, RNAs and proteins, the reassociated. Ribosomes need no help in this process.

C. Genetic code is a set of three base code words or codons in mRNA that instruct the ribosome to incorporate specific amino acids into a polypeptide. Each base is part of only one codon. It has no gaps.

D. The code is also degenerate; more than one codon can code for one amino acid. Part of the degeneracy of the code is accommodated by isoaccepting species of RNA that bind the same amino acid but recognize different codons. The rest is handled by wobble, in which the third base of a codon is allowed to move slightly from its normal position to form a non- Watson- Crick base pair with the first base of the anticodon. Such as I-U, I-C, I-A (I= inosine) Also G-U, G-C.

E. Initiation consists of binding the first aminoacyl tRNA tot he ribosome mRNA complex. Base pairing in between two regions of RNA help the ribosome recognize and bind to the initiation regions of the mRNAs.

F. Elongation has three steps:

1. EF-Tu binds an aminoacyl tRNA to the ribosomal A site.
2. Peptidyl transferase forms a peptide bond between the peptide in the P site and the newly arrived aminoacyl tRNA in the A site. This lengthens the peptide by one amino acid and shifts it to the A site.

3. EF-G translocates the growing peptidyl tRNA to the P site. Peptidyl transferase activity apparently resides on the large rRNA.

4. Translation release factors RF-1 and RF-2 recognize the stop code UAA, UAG, and UGA and cause termination.

XIV. Equation-

A. To find amount of certain nitrogenous base in a given sample.

1. X = purine Y = pyrimidine

2. X + Y = 1

3. .40 + Y = 1

4. Y = .60

5. /2 gives each base.

This discussion correlates with Lab I – DNA Isolation

Mendelian Genetics (Chapter Two)

I. Gregor Mendel- Austrian monk. Experimented with breeding at monastery in Brun using pea plants. Self taught. Farming background, tended fruit trees for a manor. Entered monastery at 21. First to discover how ratios of offspring classes revealed transmission of distinct "elementen" or characteristics by segregation of alleles at a gene. Little influence. Gave presentation of work for a few scientists at the time. Work not recognized until after his death in 1884. Other scientists used his findings in 1900. Marked beginning of modern genetics.

A. Mendel was lucky in his choice of plants. Pisum sativum. True breeding line- parental plants reproduce offspring with all the same characteristics as themselves. Through self fertilization of F1 garden pea plants obtained from

crosses between plants with different characteristics, he observed a 3:1 ratio of dominant to recessive phenotypes in the F2 progeny.

B. Pea plant experiment:

1. Seven basic characteristics:

a) yellow x green

b) white seed x gray seed

c) inflated x constricted

d) yellow pod x green pod

e) axial flower x terminal flower

f) tall x short

(Show Punnett square and ask class to explain transmission and ratio)

2. Sent his work to botanists who viewed it as too radical for the time. Sent to Hugo de Vries, Erich von Tschermak in 1866. His work paralleled theirs. Repeated work in 1900-1906 and confirmed discoveries. Rediscovered Mendel's work around 1500 – deVries, Von Tschermak, Correns.

II. Mendel's Laws

A. First Law- Gene segregation; During meiosis, homologous pairs of chromosomes separate from one another and are packaged into separate gametes.

1. (Show Punnett square and explain genotypic ratio, phenotypic ratio.)

B. Second Law- Law of Independent Assortment; A gene on one chromosome does not influence the transmission of another gene on another chromosome.

1. (Show Punnett square of dihybrid cross. Explain how Punnet square and probability can follow law of independent assortment. Show probability and Chi square formulas. Pitch fork method, triangle methods, quick math method.)

C. Basic theories of probability:

1. Probability – The likelihood of an event, based on the ratio between its occurrence and the average number of cases favorable to its occurrence taken over an indefinite series of such cases, or simply the operation of chance.

2. Sum rule – the probability of either of two mutually exclusive events occurring is a sum of their individual probabilities.

3. Product rule – the joint probability of two events occurring is the product of their individual probabilities.

4. Theory of conditional probability – the probability of a specific event divided by the probability of a more general event.

5. Binomial formula – figures the probability of x events of a particular type out of a total N.

6. Human Pedigree – Show pedigree chart with symbols and explanations. Pedigree chart shows inheritance patterns. Demonstrate carrier, infected, calculate probability. Combine Punnett Square, probability (Chi-square, pitch fork calculations), with a pedigree to demonstrate inheritance patterns. Example – use a recessive trait i.e. albinism, to easily display. Use a dominant trait i.e. Huntington's disease.

7. Work tables of observed effect (O), expected effect (E), and deviation (O-E). Work Chi-square and binomial expansion; (a+b)4=a4 + 4a3b + 6a2b2 + 4ab3 + b4 taken from (a + b)n =1.

This discussion correlates with Lab IV – Probability, Binomial Expansion, and Chi-Square.Chapter Three- Extensions and Applications

Extensions And Applications (Chapter Three)

I. Definitions;

A. Complete Dominance- mutant type of allele will always be dominant over the wild type. i.e. achondroplasia (dwarfism) and Huntington's disease. The albino allele in humans does not produce functioning gene product, and when an individual is homozygous for this allele, the biochemical pathway for the pigment melanin is blocked.

B. Incomplete Dominance- all genotypes have different phenotypes, with the heterozygotes' phenotype intermediate between the two homozygotes. Red flower x white flower = pink flower. Neither parent's phenotype was fully expressed. Could not be seen, could be biochemical differences.

C. Codominance- traits of both alleles are observed in the heterozygote. Blood groups. Type AB blood group has antigens from two different alleles.

D. Lethal Alleles- recessive resulting in the death of the homozygote. Exception- Huntingtons is dominant. Cuenot experimented with mice coat color in accordance with Mendelian crosses. Discovered yellow was dominant but that crosses between two yellow mice yielded a 2:1 ratio of yellow to wild type rather

than 3:1 expected. When crossing yellow to a recessive wild type, he found all yellow mice produced wild type progeny. He concluded yellow mice were heterozygotes and that there were no yellow homozygotes. Homozygosity for yellow was a lethal allele that died in utero.

E. Deleterious or detrimental traits- genes that affect the rate of mortality, but do not cause 100% mortality. Different rates, natural selection usually excludes.

F. Pleiotropy- single gene affects two or more characteristics. Phenylketonuria (PKU) homozygous for a defective recessive allele. Phenylalanine levels are higher in an affected individual. Can lead to mental retardation and small heads. Can be treated with a low Phenylalanine diet from birth. Some research is finding out if a pregnant woman that had PKU at birth goes back on the diet, offspring may have a lower rate of acquiring PKU. The gene that affects seed shape in garden peas also affects grain starch morphology.

G. Penetrance- a given genotype may or may not show a given phenotype. Level measured as the proportion of individuals with a given genotype who exhibit a particular phenotype. Proportion of individuals with a given phenotype within a population.100% Penetrance – phenotype always expressed. 80% Penetrance – 8 out of 10 individuals express the phenotype. Incomplete penetrance – complete penetrance. Penetrance can cause a gene to skip a generation in a pedigree.

H. Expressivity- given that a particular genotype exhibits the expected phenotype the level of expression may vary. A disease may be more severe in one person than in another. The range of phenotypes resulting from a given genotype. The

degree of expression. Variable expression, interaction with other genes and or non-genetic factors in the environment can be results.

I. Conditional lethals- genes that show their lethal effects in extreme environmental situations. The fruitfly manifests lethal alleles at variable temperatures. Primroses grow red flowers at 24 C and White flowers at 32 C. Siamese cats have darker fur on their extremities due to lower body temperatures.

J. Modifier genes- genes that have a secondary effect on a trait. Coat color in animals can vary. Effects dominant gene.

K. Phenocopy- environmental factor induce a particular abnormal phenotype that would usually be genetically determined. Disrupts normal development. Thalidomide babies.

L. Epistasis- interactions of alleles at different loci to influence a trait. Genes influence each other. The presence of two dominant alleles affect the outcome. Experiment with white flower sweet peas crossing yielded purple flower F1 generation. F1 self fertilized and yielded 9:7 ratio of purple to white. Reason – the homozygosity of recessive alleles of two different genes could result in white flowers. When both genes had one or more dominant alleles, the phenotype was purple.

1. Complementary gene action- type of epistasis that requires the interaction of genes to jointly produce a specific gene product, i.e. white and purple flower example above.
2. Duplicate gene action- type of epistasis when either of two genes function to produce the dominant phenomenon. The two different genes

produce similar gene products and one of them may have arisen by duplication from the other gene. i.e. Shepherd's Purse weed. Round seed pods result when dominant alleles are present at either of two genes, while narrow seed pods are produced only in the absence of dominant alleles at both loci.

M. Genomic imprinting = expression of an allele (gene) may differ due to whether it was inherited from the mother or the father. Asthma, retinoblastoma, Huntington's disease. Angelman Syndrome (mother) severe retardation, puppet like movements, uncontrollable laughter. (15QIIQ13) Prader-Willi Syndrome (father) retardation, obesity, insatiable appetite. *Chapter 15 discusses DNA methylation, touch on briefly here, go into detail in a later chapter.

1. reversible alteration through inheritance
2. restricted to certain segments only
3. not a mutation
4. not a permanent change
5. only affects gene expression
6. each generation erases imprinting before meiosis.

N. Sex-Linked:

1. X-Linked – traits determined by genes on the X chromosome. There are 161 loci on the X chromosome. Color blindness is caused by a recessive allele on X. Males are XY, if they have the recessive gene on their X, they are colorblind because Y does not have this loci. X-linked usually has a pattern throughout inheritance which helps distinguish it

from autosomal. Affects more males than females, XY to XX. No offspring of an affected male are affected, making the trait skip generations, always with an unaffected female in the intermediate generation. i.e. Color blindness, hemophilia.

2. X-linked dominant – Affected males produce all affected female offspring and no affected male offspring. Approximately half the offspring of affected females are affected, regardless of their sex.

3. Y-linked – Trait is always passed from father to son, only males are affected. McKusick lists fewer than 20 anamolies. i.e. Hairy ear rims.

4. Sex Limited- Traits that are expressed in only one of the two sexes. Mostly relating to reproductive characters such as milk yield in cattle, or egg laying in chickens.

5. Sex Influenced- Genotypes determined by autosomal genes are expressed differently in the two sexes. i.e. Traits that determine horn formation in sheep, in breeds that both sexes have horns, males are larger than females. Premature baldness in humans (before age 35) follows this suit. Dominant in males, recessive in females.

6. Inborn errors of metabolism – enzymatic defects that result in the buildup or absence of an important biochemical product. A large number of autosomal inherited genetic disorders are caused by an inborn error of metabolism.

This discussion correlates with Lab V – Drosophila Culture

Chromosomes And Heredity (Chapter Four)

I. Chromosome etiology.

A. Chromosomes are characterized by size, shape, location of the centromere and banding.

B. P arm; top arm, smaller than bottom

C. Q arm; bottom arm, larger than top arm

D. Centromeres:

1. Place on the chromosome where spindle fibers are attached during cell division. Primary constriction.

2. Kinetochore- structure around the centromeric region.

3. One centromere = 1 chromosome

4. Metacentric- centromere is located roughly in the center, separating the two arms into almost equal length.

5. Submetacentric- centromere is off center resulting in the bottom arm being longer than the top arm.

6. Acrocentric- centromere is off center. The top arm has a secondary constriction resulting in satellites on the end of the P arm. Secondary constriction usually contains the information needed for the nucleolus.

7. Telocentric- centromere is located very near the terminal end of the P arm.

8. Telomere – structure at the end of a eukaryotic chromosome, containing tandem repeats of a short DNA sequence. Sequence is different from the rest of the chromosome.

II. Chromosome-

A. Female 46 XX no sex determinants on X chromosome. Male 46 XY Y chromosome only responsible for sex determination. One X in the female is activated. Female needs both XX to be female.

B. X Inactivation – Very tightly coiled. Happens in early development. XIST gene is responsible for inactivation. Protein from gene activates X. This occurs in females. X inactivation is not random.

C. Reactivation – reactivation occurs before meiosis in ovary before gametogenesis.

III. Karyotyping and Cytogenetics-

A. Karyotyping- pictorial or photographic representation of all the different chromosomes in a cell of an individual, usually sized and numbered from greatest to smallest.

1. Procedure for Karyotyping:

a) Sample of cells is taken, could be peripheral white blood cells(lymphocytes), amniotic fluid, chorionic villus sampling, bone marrow, solid tissue (tumor, skin)

b) Cells are cultured by use of a culture media (nutrient). Mitogen is used to induce cell division. PHA, phytohemme aglutinin, is used to clump red blood cells and stimulate their division.

c) Spindle fiber inhibitor, colcimid or colhegin, stops the division at metaphase.

d) Cells are harvested by inducing hypotonic shock, discovered by Dr. T.C. Hsu.

e) Slides are prepared.

f) Samples are stained using Wright stain (good to count the number of chromosomes) or Geimsa stain, which shows banding of the chromosomes. G-Banding= Sample is treated with trypsin, then giemsa, the bands show up clear on each chromosome.

g) Photograph of the cell is taken. Chromosomes are then arranged by size order.

IV. Reason for chromosomal analysis

A. Multiple congenital anomalies

B. Mental retardation/developmental delay

C. Abnormal or delayed sexual development

D. Multiple spontaneous abortions

E. Amniocentesis or advanced maternal age

F. Malignancy

V. International System for Cytogenetic Nomenclature

A. Denver system of classification. Conference in Denver Colorado in 1960 to decide naming of groups of chromosomes. Determined 1-7. London conference in 1963 changed to A-G.

B. Groups A= first three pairs of chromosomes. Easy to identify due to drastic difference in length of arms and location of the centromere. Chromosome number 1 often has a secondary constriction.

C. Group B= chromosomes 4 and 5. Shorter than Group A, with subterminal centromeres.

D. Group C= chromosomes 6-12 and the X chromosome. Chromosomes 6, 7, 8,11, and X are metacentric. Chromosomes 9, 10, and 12 are submetacentric.

E. Group D= Three chromosome pairs that are difficult to distinguish. They are of equal length with centromeres in an acrocentric position with satellites.

F. Group E= chromosomes 16, 17, and 18. Number 16 sometimes will have a secondary constriction in the proximal portion of the long arm.

G. Group F= chromosomes 19 and 20. Undistinguishable.

H. Group G= chromosomes 21, 22, and Y. Number 21 and 22 have satellites. Y chromosome has long arms that lie very close to each other.

VI. Chromosome Structure

A. Landmark = a morphological feature on a stained chromosome.

B. Region = an area between two adjacent landmarks on a chromosome.

C. Band = a distinctive dark or light line on the chromosome.

D. Sub-Band = band within a band. Look closely at a dark band, you can sometimes see areas of dark and light. These are sub-bands.

E. Demonstrate by slides, transparencies the structure of a chromosome and show landmark, region, band, and sub-band. Display numbering from centromere to terminal end. Show p and q arm.

VII. Nomenclature

A. When numbering a chromosome region, start at 1 at the centromere and number out to the terminal end. True for both arms.

B. 46XX (del (7)) P21.3 = 46 chromosomes, female, deletion on chromosome number 7, P arm, region 2, band 1, sub-band 3.

C. 47XY(+21) Downs male

D. 7Q 31.2-31.3 Chromosome 7, Q arm, deletion from region 3, band 1, subband 2 through region3, band 1, sub-band3. Cystic fibrosis

E. Break in a chromosome is notated : A break with a join is notated :: (translocation). Interstitial translocation involves the one-way movement of a segment. Reciprocal translocation involves two way exchange of chromosomal segments.

This discussion correlates with Lab X – Human Genetics.

VIII. Genes and Chromosomes:

A. Morgan- first definitive study that genes and chromosomes are related.

B. Nondisjunction- abnormal separation of chromosomes during meiosis. i.e. fruit fly eye color in Morgan's experiments.

C. Duplication- When a chromosomal segment is represented twice. Categorized by the position and order of the duplicated region.

1. Tandem duplication – duplication is adjacent to the original chromosomal region, same as the original. Homologs overlap and break in different points. If the different homologs reunite, one chromosome will have a tandem duplication and the other a deletion of the duplicated area. The duplication and the deletion are reciprocals of each other.
2. Reverse duplication – opposite order of a tandem duplication.

3. Displaced duplication – duplicated region not adjacent to the original segment. The displaced duplication may still be on the same chromosome or on another.

D. Deletion – missing chromosome segment.

1. interstitial deletion – an internal part of the chromosome is missing.

2. Terminal deletion – only one break and the homolog fails to rejoin. The tip of the chromosome is usually lost in cell division because it does not have a centromere.

3. Homozygous deletions are lethal due to the fact that they are missing essential genes.

E. Inversions – Most chromosomes have the same sequence to their genes. Inversions occur when the sequences do not match. They can be generated by a simultaneous break at two points in a chromosome and followed by an incorrect union. Individuals heterozygous for inversions can be distinguished by the presence of inversion loops in meiotic pachytene chromosomes.

1. Paracentric inversion – When the inverted segment does not contain the centromere.

2. Pericentric inversion – when the inverted segment does contain the centromere.

F. Translocation – movement of breaking and joining of a chromosomal segment from one chromosome to another, nonhomologous chromosome.

1. Interstitial translocation – involves one way movement of a segment.

2. Reciprocal translocation – involves a two-way exchange of chromosomal segments. (More common) If the two segments of a reciprocal are large and the others are small, the smaller chromosomal regions are lost, reducing the number of chromosomes.

IX. Chromosomal Abnormalities :

A. Abnormalities occur .75 – 1% of the population.

B. Euploid – chromosome number sets differ.

1. Polyploid – three or more complete sets of chromosomes.

a) Triploidy - Three sets. 69 chromosomes, an entire extra set. Fusion of fingers, abnormal features, heart disease, die soon after birth. Reason – Polar body stays or two sperm hits the egg at the same time.

b) Tetraploidy – Four times the normal number of chromosomes. Normal features, look similar phenotypically to a Trisomy 18. Reason – First mitotic division did not occur.

C. Aneuploidy – too many chromosomes, not diploid.

a) Trisomy – gamete with an extra chromosome 2n +1.

b) Monosomy – gamete missing a chromosome 2n –1. Turner Syndrome, dema sack in neck, short stature

D. Down Syndrome – Trisomy 13, Trisomy 14 are inherited translocation. 13 – Die soon after birth, big nose, closed eyes, polydactylism. Trisomy 18 – "Classic Trisomy" small pointed ears, heart defects, clench hands. Trisomy 21 is not inherited.

Genetic Linkage (Chapter Five)

I. Exceptions to Mendel's principle of Independent Assortment were discovered in 1900's. Alleles at different genes on parental chromosomes tend to remain together because of the distance between genes on a chromosome.

A. Coupling – Physical connection between the parental alleles, the dominant from one parent and the recessive from the other. Interferes with independent assortment.

B. Repulsion – negative affinity for a dominant or recessive allele from one parent or the other.

C. Recombination (crossing over) – the physical exchange through which new chromosomal types are formed. Indicated in diagrams by an "x" joining homologous chromosomes.

D. Linked genes (linkage)– genes on the same chromosome. Originally linkage of humans was done by accumulating information from informative matings. Later done by somatic cell hybridization, discussed below.

E. Linkage map (genetic map) – used to describe the physical relationship of genes on a chromosome in a linear arrangement. Uses the frequency of recombinant gametes (offspring) from a testcross as a measure of the distance between two genes. Morgan and Sturtevant suggested that distance along the chromosome be measured in units (map units) determined by the percentage of recombination. Similar to cytologically or biochemically measured distances on a chromosome. Distance measured from 0, tightly linked, to ½ , for loosely linked.

There is a close correspondence between the genetic map distance and the physical DNA distance.

F. Interference – Method dividing the observed numbers by the expected numbers of double crossovers and subtracts it from one unity. Gives the measure of interference of one cross over on another.

G. Deletion mapping – Technique through which genes can be located to a particular chromosome segment. Example: A heterozygous deletion on a given chromosome that has a number of codominant genes (blood groups) if different alleles are present at each gene, each genotype should be recognizable as heterozygote. However when a gene is in the deleted region, a heterozygote should always appear as a homozygote.

H. Somatic cell hybridization – Cells from two different species are fused in the lab using a fusing agent like Sendi virus. Sendi virus has several attachment to a host. Traits are monitored over time (humans are usually mixed with hamster). Eventually human chromosomes are lost, different cell lines lose different human chromosomes. The chromosomes that contain the gene of interest is determined by noting which cell lines still carry the trait and finding a human chromosome they have in common.

This discussion correlates with Lab XII – Pedigree Analysis

Gene Mutation (Chapter Eleven)

I. Germ-line Mutation – Multicellular organisms that reproduce sexually can experience mutation in their sex cells, in which case the gametes can be altered and the mutation

passed on to the progeny. Queen Victoria had a germ-line mutation, all her male heirs were afflicted with hemophilia.

II. Somatic mutations – (non sex cells) may change the phenotype of the individual that suffers the mutation, but the mutant trait will stop with that individual. The mutation does not affect the gamete so it is not passed on. Example – cancer, derived from a single cell that has changed its behavior from normal to malignant.

III. Morphological or visible mutation – can be determined from wild type organisms because of their altered appearance. Example – albino mammals have a mutation in a gene that is responsible for dark color pigment. A mutated tyrosinase gene may produce no active enzyme, so no melanin can be made.

IV. Lethal mutations – a mutation so severe that the individual carrying it can not survive. i.e. inactivating mutation in the gene for one of the subunits of RNA polymerase. If the mutation rendered the RNA polymerase inactive, no RNA could be made, therefore the organism could not live. Haploid organisms with lethals die immediately because they do not have a wild type gene to compensate. Diploid can carry a lethal because of their wild type masking ability. Huntington's is an example of a dominant lethal. It is still not known how someone can live with a dominant lethal and pass it on, we do know that heterozygotes live well past childhood years and do not show symptoms until later life.

V. Conditional mutations – mutations that are lethal in certain conditions.

A. Temperature sensitive mutations – allows growth at low temperatures. i.e. Siamese cat. These animals have a mutation in the gene for dark coat color. The dark color is visible on areas of their body where the temperature is lower than others, feet, faces, and ears. Most of the cats coat is warm enough to inactivate the

color-producing enzyme, so it is white, but the extremities are cool enough to produce the dark color.

VI. Point mutations – involve the alteration, insertion, or deletion of one or a few bases at a time.

A. Missense mutations – base change alters the sense of a codon from one amino acid to another. This causes an improper amino acid to be inserted into the protein product of the mutated gene. Example – sickle cell anemia. When oxygen is depleted in the blood, their red blood cells change shape into a sickle which can not fit through a capillary and therefore starves the body of blood.

VII. Mutagen – mutation causing agent.

VIII. Mutators – replicated strands with more mistakes than normal, mutation rates will be higher. These have been mapped to several different genes. There are also mutations that make DNA replication even more faithful than normal. If a more faithful replication of DNA is available, why have E. coli evolved with a less effective one? Mutants with these extra-faithful system have too slow a rate of evolution; they are flexible enough to compete with more changeable organisms in adapting to a shifting environment.

IX. Frameshift mutation – sometimes DNA replication causes the insertion or deletion of one or more bases in the middle of a coding region which changes the translational reading frame from that point on. They are very severe because they change every codon from the point of the mutation to the end of the mRNA. Premature termination is often the result.

X. Oxidative Deamination – bases, especially cytosine, have the tendency to lose their amino groups in this process. A deaminated cytosine receives a carbonyl oxygen in place

of an amino acid group converting it to uracil which will base pair with adenine instead of guanine. Most often does not lead to mutation because cells have a mechanism for removing uracils that find their way into DNA by mistake.

XI. Chemical mutagens-

A. Electrophilic – some natural and synthetic substances in our environment are electrophilic, negative charge loving. Electrophiles seek centers of negative charge in other molecules to bind to. Good example – DNA. Every nucleotide contains one full negative charge on the phosphate and partial negative charges on the bases. When attacked by electrophiles, they add carbon-containing groups called alkyl groups. This process is alkylation. Favorite attack sites are N7 of guanine and N3 of adenine. They then make the bond between base and sugar easier to break. Replication, if it does occur, does not occur correctly causing a mutation.

XII. Reversion (back-mutation) – usually in frameshift mutations, can occur in two ways: true reversion – alteration of the mutated base back to its original identity, or a change elsewhere in the same gene to compensate for the original mutation. Suppression – second- site reversion – compensation by one mutation for the effects of another. Intragenic suppression- both mutations occurring in the same gene. Intergenic suppression – suppression of one mutation in one gene by a mutation in another. Since it is on two different genes, it is not considered reversion.

XIII. DNA repair

A. Kelner 1940's experiments with UV radiation damage. Photoreactivation, or light repair is catalyzed by an enzyme called photoreactivating enzyme or DNA

polymerase. This enzyme operates by first detecting and binding to the UV damaged section (a pyrimidine dimer). Then the enzyme absorbs visible light, which activates it so it can break the bonds holding the pyrimidine dimer together. This restores the pyrimidines to their original independent state. Finally, the enzyme dissociates from the DNA and the damage is repaired.

B. Excision repair – The damaged DNA is first removed then replaced with fresh DNA. This is done by one of two mechanisms:

1. Base excision repair- Certain mutations are recognized by an enzyme called DNA glycosylase, which breaks the glycosilic bond between the damaged bases and its sugars. This leaves an apyrimidinic site (AP site) which is a sugar without its base. The AP site (also a purinic site) is recognized by AP endonuclease that nick the DNA strand on either side of the AP site, removing the AP sugar phosphate. DNA polymerase I fills in the gap by inserting nucleotides to pair with the ones in the opposite strand. DNA ligase seals the nick to finish the job.
2. Nucleotide excision repair – The incision makes the cuts on either side of the mutation, removing oligonucleotides with the damage. The key enzyme is uvrABC endonuclease and it contains three polypeptides. A more general term for the enzyme system that catalyzes nucleotide excision repair is excinuclease.

C. Coping with DNA damage without repair. There are ways cells cope with damage without having to fix the problem. These means are called repair mechanisms, even though there is no repair.

1. Recombinant repair – is the most important mechanism because it requires DNA replication before it can operate. This creates a problem for DNA with pyrimidine dimers because the dimers stop replication. When replication does occur, there is a gap across from the dimer. Recombination occurs between the gapped strand and its homologue on the daughter DNA duplex. The net effect is to fill in the gap across from the pyrimidine dimer and to create a new gap in the DNA duplex. Since the other duplex has no dimer, the gap can be easily filled in by DNA polymerase and ligase. The DNA damage still exists, but the cell has managed to relicate.

2. Error prone repair – This process entails inducing the SOS response which causes the DNA to replicate even though the damaged region can not be read correctly. This results in errors in the newly made DNA.

D. DNA repair defects in humans; Xeroderma pigmentosum, Fanconi's anemia, Bloom's syndrome.

This discussion correlates with Lab VI – Sex Chromosomes and Gene Transmission.

Transposable Elements (Chapter Twelve)

I. Transposable elements – (transposons) Pieces of DNA whose primary function is their own replication that move around in prokaryotes and eukaryotes. They can insert copies of themselves throughout the genome and can cause rearrangement of host DNA giving rise to mutations. "Jumping Genes"

II. Conservative transposition – mode of transportation where DNA leaves one place and jumps to another. Both strands of DNA are conserved and move together.

III. Replicative transposition – one copy of the transposon remains at its original site and a copy moves to the new site.

IV. Mu – bacteriophage that can act as a transposon. Its replication depends on transposition.

V. Retroviruses; Reverse transcription – RNA to DNA, SS. RNA –DS. DNA. Catalyzed by an enzyme called reverse transcriptase or RNA dependent DNA polymerase. Plays a major role in eukaryotic genomes.

VI. First Examples of Transposable Elements: Barbara McClintock, 1940's, corn. Indian maize color variation due to unstable mutation, wherever the mutation has reverted, the reverted cell and its progeny will be able to make pigment, giving rise to dark spots on the kernel. Lots of dark spots mean the mutation was unstable: it reverts at a higher rate than that of an ordinary mutation. McClintock discovered the original mutation resulted from an insertion of a transposable element called Ds for "dissociation". Ac is for "activator" that causes reversion. Ds can transpose, but only with the help of Ac, Ac can transpose itself and therefore inactivate other genes without help from other elements.

VII. Best Studied Eukaryotic Transposons: yeast and fruit flies. In Drosophila, the transposon is called copia, due to the fact it is evident in copious quantity. Copia and copia-like elements account for 1% of the total fruit fly genome. Similar transposons in yeast are Ty, "transposon yeast".

A. Physical structures in both are similar. They contain genes that are necessary for their own transposition and they cause duplication of the host DNA at either end.

B. Differences: Ty contains 330 base pair direct terminal repeats called delta repeats. Ty transposes by a pathway very differently from the one taken by bacterial transposons. It involves an RNA intermediate and is very similar to the replication scheme of eukaryotic viruses called retroviruses.

C. Retroviruses: Some cause tumors, some cause AIDS: HIV is a retrovirus. Retroviruses can be considered transposons in that their genomes, (or DNA copies of their genomes) are found inserted randomly into host DNA. They have the ability to make a DNA copy of its RNA genome. This reaction, RNA to DNA, is the reverse of the transcription reaction, so it is commonly called reverse transcription. The virus particles contain an enzyme that catalyzes the reverse transcription reaction, RNA dependent DNA polymerase.

D. Cycle of retrovirus replication: Start with an infected cell. The cirus contains two copies of its RNA genome, linked together by base pairing at their 5' ends. When the virus enters a cell, its reverse transcriptase makes a double stranded DNA copy of the viral RNA, with long terminal repeats (LTRs) at each end. This DNA recombines with the genome host to yield an integrated form of the viral genome called a provirus. Host RNA polymerase II makes viral mRNAs, which are then translated to viral proteins.

VIII. Retrovirus-like Transposition of Ty: Several mpieces of evidence that Ty transposition resembles replication of retroviruses;

A. Ty encodes a reverse transcriptase. The tyb gene in Ty codes for a protein with an amino acid sequence closely resembling that of the reverse transcriptase encoded in the pol genes of retroviruses. If the Ty element codes for reverse transcriptase, then the enzyme should appear when Ty is induced. Also, mutations in tyb should block the appearance of the reverse transcriptase.

B. Full length Ty RNA and reverse transcriptase activity are both associated with particles that closely resemble retrovirus particles. These only appear in yeast cells that are induced for Ty transposition.

C. When an intron was inserted into a Ty element and then analyzed. The intron had disappeared. This is consistent with the following mechanism: The Ty element is first transcribed, intron and all; then the RNA is spliced to remove the intron; and the spliced RNA is reverse transcribed within a viruslike particle and the resulting DNA is inserted back into the yeast genome at a new location.

This discussion correlates with Lab X – Human Pedigree

Genetics of Bacteria and Phages (Chapter Thirteen)

I. Bacterium goes through three phases of growth in a culture

A. Lag phase – bacterium divides slowly because they are adjusting to life in new conditions

B. Log phase – bacterium divides exponentially

C. Stationary phase – growth stops due to exhaustion and accumulation of toxic intermediates of their medium.

II. Bacteria grown on –

A. Agar – nutrient surface of seaweed.

B. Minimal medium – salts and sugar glucose, lets the bacteria make their own amino acids and vitamins. Ones that produce all are called prototrophs and those that do not are auxotrophs.

III. Bacterial transfer of DNA – 1946 Lederberg and Tatum. Hypothesis: Bacteria can exchange DNA info. Procedure – crossed two different mutant strains called auxotrophs and look for wild type recombinants. Chose E. coli strain K12, which does exchange info. Conclusion – DNA from the chosen auxotrophs mixed and recombined, but it did not show conclusively that DNA transfers directly from one cell to another. One auxotroph could have burst and its DNA could have transformed from the other. To rule out, one strain was lysed and filtered to remove whole cells. Then cell-free filtrate containing DNA to see if it would transform the other. It did not. Conclusion – two strains of bacteria in the original experiment had transferred DNA from one to another by direct contact (conjugation).

A. Conjugation is made possible by a tubular structure called a sex pilus on the surface of the strain. Modern term: F pilus for "fertility".

B. One way DNA transfer during conjugation: Despite all the sexual nomenclature in fertility of bacteria, these organisms rarely transfer their entire genomes to form a true diploid zygote. They form partial diploids called merodiploids or merozygotes. Conjugations has an advantage- it allows them to form recombinant organisms with new combinations of alleles not present in the parents.

C. Hfr strains: Late 1950s discovered new E.coli strain in addition to F+ and F-. Stands for "high frequency recombinant". These are mutant F+ strains that have lost their ability to transfer fertility at high frequencies. Instead they transfer host chromosomal genes. An F+ cell will convert to an Hfr when its F plasmid inserts into the host chromosome. Two reasons why F+ contain Hfr:

1. an equilibrium between F+ and Hfr cells such that each F+ spends a small fraction of its life as an Hfr.
2. F+ cells may occasionally mutate to Hfr cells. Mutation being permanent.

IV. F plasmid insertion:

A. An F plasmid can insert into a host chromosome because of the similarity between transfer of the F plasmid and transfer of the host genes. Occasionally the F plasmid recombines with its hosts' chromosomes converting the cell to Hfr. This mobilizes the Hfr cell's chromosome to transfer to recipient cells. Think of a rolling circle style replication of the Hfr chromosome, with one daughter DNA molecules entering the recipient cell and recombining with its chromosome to change its genotype.

Developmental Genetics (Chapter Fourteen)

I. Definitions:

A. Differentiation – process a cell goes through during development so they will be able to manufacture specialized products like insulin or hemoglobin. All cells,

even though they go through specialization, retain and continue to make all the key proteins essential for life.

B. Housekeeping genes – genes that function for maintenance, ribosomal proteins and RNA polymerase.

C. Luxury genes – genes that specialize for hemoglobin or insulin. These are "luxury" only in that their products are not immediately vital to the survial of the cells that make them, but they are needed for the entire organism.

D. Mosaic embryo – embryo that produced genetically dissimilar cells. Example – Styela; At the two cell stage, the two embryonic cells (blastomeres) are different. They continue to be different in the four cell stage. Roux theory – embryonic cells begin losing genetic potential from the very beginning, so that each cell division during development involves the unequal partitioning of genetic material between the daughter cells.

E. Totipotent – cell that has the ability to become any type of cell in the organism.

F. Regulative embryos – when embryonic cells remain similar for longer periods of time. Humans and frogs remain similar up to the first three divisions, proof due to identical quintuplets.

G. Determination – Before a cell differentiates, it expresses its specialized function. Process – stage at which the cell passes the point of no return in its specialization. Determinants – Cytoplasmic effector substances.

H. Inducer – substance that one cell makes to influence the development of another cell. Can produce wrong effects if signaled to the wrong address.

I. Morphogens – subclass of determinants and inducers that act to determine shape in a developing embryo.

J. Retinioc acid receptor – nuclear receptor which binds to their respective ligands (hormones) and then activate sets of genes by binding to nearby enhancers known as hormone response elements (HRE). Target to RAR is retinoic acid response element (RARE).

K. Gap genes – zygotic genes which produce embryos with missing parts or gaps where structures would normally be.

L. Selector genes – group of genes which determine the development of cells into structure or characteristics of each parasegmental compartments. The genes switch on to guide cells down a particular developmental pathway and remain on to lock cells permanently into the pathway.

M. Signal transduction pathways – the activity of a gene in one cell can turn on or off genes in another cell.

N. Epistasis – the alteration or masking of the phenotype of one mutation by a mutation in another gene. Geneticists use this phenomenon to discover whether or not two or more genes participate in the same pathway.

II. Antibodies- proteins that are produced inr esponse to an antigen (foreign substance that will elicit the production of an antibody)

A. Composed of four chains of protein (polypeptides); 2 long and 2 short (amino acids)

B. Long chain is the heavy chain (4 separate genes)– 446 amino acids

C. Short chain is the light chain (3 separate genes)– 214 amino acids

D. Joined by disulfide bonds

E. “Y” shaped molecule

F. Constant and variable regions – Variable = light, constant = heavy.

G. Neutralize the effects of an antigen by lysis, clumping, phagocytosis, opsonization (coating the virus)

III. Classes of Immunoglobin

A. IgM – secreted first within a week of life. Large molecule (5 units) 7%.

B. IgG – secreted later, secondary immune response that will elicit macrophage that will attack small molecules. Can cross the placenta. 80% of Ig in blood is IgG

C. IgE – associated with inflammatory response; histamine, allergies, cancer(?). Small amounts (trace).

D. IgA – Provide immune protection to nursing babies; present in fluid- saliva, milk, tears, urine. 12%

E. IgD – Function relatively unknown – small amount.

IV. Immune System Defenses –

A. Cell mediated immunity response – T lymphocytes; directed against body cells

B. Humoral immune response – B lymphocytes (AB) mediated by an antibody.

C. Acquired immunity – develop after birth

D. Innate immunity – born with the immunity

V. T lymphocytes – Bone marrow = thymus

A. Thymus – multilobed organ behind breast bone

B. Place where T cells are matured and modified

C. Becomes immunocompetent (T cell education)

D. Can learn to distinguish self from non self.

VI. B lymphocytes –

A. B = bursa of chicken or its equivalent (bone marrow, appendix, lymph gland)

B. B cells = Plasma cells

C. Plasma cells produce antibody (Immunoglobulin, gammaglobulin)

VII. Process –

A. Bone marrow – Stem cells – Thymus – T cells – Peripheral cells – activation – helper, suppression – killer, inducer – interferon and interleukins.

B. Lymphoid tissue (appendix or tonsils) – lymph gland – B cells – Peripheral B cells – activation – Plasma cells – all five antibodies and memory cells.

1. Active immunity – make the antibody yourself
2. Passive immunity – antibody placed in body.

VIII. Genomic imprinting – Gene expression is based on whether the gene is inherited from the mother or the father. Follows non-Mendelian inheritance. i.e. Prader-Willi syndrome and Angelman Syndrome in humans. Both from inheritance of chromosome 15, decided from whether inherited from mother or father. Reason – DNA methylation. Genes from male and female are methylated differently, studies show that this methylation can be reversed. The methylation of a gene can decide if the gene is expressed. Advantage – IGF insulin-like growth factor, makes babies bigger, advantageous to life. Mother repressed due to size of baby, father's genes live on. Battle of sexes at the molecular level. *Show examples of PWS and AS, methylation diagram, etc.

This discussion correlates with Lab IX – Sex Chromatin in Human Cells.

Gene Cloning and Manipulation (Chapter Fifteen)

I. Clone – group of identical organisms. Growing an entire plant from a clipping. John Gurdon produced frogs from transplanting nuclei from a single frog embryo to many enucleate eggs, identical twins can be considered clones.

II. Procedure usually constitutes placing a foreign gene into a bacteria cell, then to grow a clone of these modified bacteria, with each bacterial cell containing the foreign gene. As long as we ensure the foreign gene can replicate, we can clone the gene by cloning its bacterial host.

III. Stanley Cohen and Herbert Boyer performed the first cloning experiment in 1973. Experiment depended on restriction endonucleases discovered by Linn and Arber in the late 1960's. These enzymes prevent invasion by foreign DNA, like viral DNA, by cutting it up. They actually restrict the host range of the virus, and cut sites within the foreign DNA instead of cutting at the ends. The result was a recombinant DNA, two previously separate pieces of DNA linked together. It was easy to detect because when introduced into bacteria cells, it conferred resistance.

IV. Vectors – All gene cloning experiments require carriers called vectors. Since the 1970's there are many new vectors, they fall into two categories:

A. Plasmids – pBR322, pUC. PBR322 has two antibiotic resistance genes and a variety of unique restriction sites into which we can introduce foreign DNA. Most of these sites interrupt one of the antibiotic resistance genes, making selection

straightforward. PUC have an ampicillin resistance gene and a multiple cloning site that interrupts a partial b-galactosidase gene.

B. Phages – Alpha has certain nonessential genes removed to make room for inserts. Some can accommodate inserts up to 20 kb which make them useful for building genomic libraries. Cosmids can hold up to 50 kb. The other phages consist of M13 which have the convenience of a multiple cloning site and the advantage of producing single stranded recombinant DNA. This DNA can be used for DNA sequencing and site directed mutagenesis. Plasmids called phagemids have also been engineered to produce single stranded DNA in the presence of helper phages.

Genes and Cancer (Chapter Seventeen)

I. Characteristics of cancer cells – Malignant cells are normal cells that have changed. Process relatively unknown. Three differences between normal and cancer cell.

A. Malignant cells are immortal, they will go on dividing forever.

B. They exhibit characteristics that contrast normal cells called transformed. Normal cells need a hard surface to grow, malignant cells can grow in soft agar. When a normal cell gets crowded, it will stop dividing. Malignant cells can pile on top of one another if they get crowded.

C. Malignant cells will lose the characteristics, biochemical and genetic, of the normal cell which they came from.

II. Causes of Cancer

A. Genetic – Mendelian, single gene defect. Characteristics = early age of onset, affected close relatives, multifactorial tumors. Genetic disease that results from accumulation of mutations, gene loss, and gene rearrangement.

B. Non- Mendelian = polygenic. Common lifestyle or environmental exposure, coincidence. Consider heredity and environment as two ends of a spectrum.

C. Carcinogens – cancer causing agent. Electrophilic compounds that attack centers of negative charge in DNA causing mutations. The mutations start the chain of events that will lead to cancer.

D. Radiation – unrepaired DNA lesions caused by ultraviolet radiation can lead to cancer. The improper repair of DNA, not the damage itself causes the cancer.

E. Virus – Oncogene (tumor causing) virus that transforms the host cell. This is descended from a related cellular proto-oncogene.

F. Proto-oncogene – important in normal cell growth, can cause a cell to take neoplastic characteristics which will result in a tumor cell. Important in normal cell growth but can cause cell to take neoplastic characteristics. Example: in tumor cells (oncogene) only one allele is necessary to produce cancer phenotype (gain of function mutation).

G. Oncogene- only one allele is necessary to produce the cancer phenotype (gain of function mutation)

H. Mechanism of oncogene –

1. point mutation
2. translocation
3. change of expression

4. create a chimeric gene
5. amplification

I. Tumor suppressor genes – normal cellular genes that regulate cell growth and development. Can slow down or turn off growth. Recessive at cellular level. Both alleles must be mutated or lost to produce cancer phenotype. (loss of function)

J. Knudson two mutation hypothesis: Heredity – first mutation is in the germ line and second mutation is in the somatic. Sporadic – both mutations are in the somatic cell.

K. Chromosome abnormalities in tumors – specific abnormalities are associated with specific kinds of cancers. Many have prognostic significance. There is a relationship to the carcigenesis and the progress.

L. Retrovirus – RNA tumor virus which replicates via a double stranded DNA intermediate. Example; HIV AIDS.

M. Helper viruses – Most transforming retroviruses lack one or more of the genes required for viral replication, they need a helper virus to replicate.

N. Amplification – Proto-oncogene activation. More gene product is made due to repeated replication.

O. Double minute chromosomes (DM's) – tiny chromatin bodies lacking centromeres and containing amplified DNA. Looked for when testing for amplification.

P. Homogeneously staining regions (HSR's) – parts of chromosomes where the regular banded staining pattern gives way to an amorphous, uniformly stained zone that contains amplified DNA. Looked for when testing for amplification.

Q. Nerve growth factor – stimulates nerve cells to grow. Receptor spans the cell membrane and the protein protruding outside the membrane binds the growth factor which activates the other part of the protein that is in contact with the cytoplasm.

This discussion correlates with Lab XI – Pedigree Analysis

Population Genetics (Chapter Nineteen)

I. Population genetics – field that focuses on the extent and pattern of genetic variation in natural populations and the explanations for these observations.

A. Gene pool – the genetic constitution of a population.

B. Population – group of interbreeding individuals of the same species that exist together in time and space.

C. Polymorphic – a gene having many forms, having two or more alleles in substantial frequency.

D. Monomorphic – having only one allele in high frequency.

E. Hardy-Weinberg Principle – relationship exists between allele frequency and genotype frequency. The genotypic frequencies for a gene with two different alleles are a binomial function of the allelic frequencies. *Demonstrate HW

F. Chi-Square Test – Test to determine if the fit is sufficiently close to the expected. X2 = E(O-E)2/E. *Demonstrate Chi Square.

G. Inbreeding – nonrandom mating that results from mating of too close relatives. Many individuals with recessive diseases may be a product of inbreeding.

H. Outbreeding – Reproducing mating by chance at random from the population.

I. Positive assortative mating – Mated pairs in a population that are composed of individuals with the same phenotype more often than would be expected by chance. Height and eye color.

J. Negative assortative mating – Mated pairs that share the same phenotype less often than expected.

K. Inbreeding coefficient - Value defined as the probability that the two alleles at a gene in an individual are identical by descent. Alleles are identical by descent when the two alleles in a diploid individual are derived from one particular allele in their ancestry.

L. Spontaneous mutation – appear without apparent explanation.

M. Induced mutation – caused by some mutagenic agent.

N. Genetic drift – Due to small population numbers by chance effects, introducing sampling gametes from generation to generation to change allelic frequencies.

Class Discussion Topics

I. This section is designed for group discussions. The purpose is to ensure students understand the concepts discussed in the class. These discussions are a type of forum for interaction between the students and the instructor. The students get a chance to demonstrate their knowledge of previous topics. An open discussion can get students to think in new ways just by listening to the views of others. This can also be used as a tool to engage in ethical discussions.

A. How is cloning accomplished?

1. Go back to cell differentiation and know that mechanism. Each cell starts out with the ability to create every cell in the body. During differentiation, a cell "covers" all the information it has except that of what cell it is to become, i.e. a skin cell still has all the info to make every cell, but only uses the info to become a skin cell. The information not needed is "covered". It is still in the cell, just not used. To clone, the "cover" over the unused info, all the info to make every cell, is lifted. To date, this technique has been used to clone Dolly the sheep, mice at the University of Hawaii, and cows.

B. What about human needs?

1. Case of Anyssa Ayala, 17 year old in California with Leukemia. She had a slow growing tumor that doctors predicted her death within three years if she did not receive matching bone marrow. She could not find a match, so her parents decided to have another child and see if the babies marrow matched her sister. The child, Marissa, was a perfect match and the operation was successful. This is a case where Anyssa's cell could have been harvested and lifted if the technology was available.
2. Ethical issues of having a child to save a child.
3. Ethical issues of harvesting own cells.

C. The Hitler issue.

1. Cloning evil to make evil argument. Personality mostly due to environment. Cloning does not make an exact clone. Genetically the same,

same cells, same genetic make-up. Difference comes in thought, personality, etc.

2. Identical twins are, at this point, our best example of clones. Share same genes, etc. Take the case of Chang and Eng (1811-1874), the Siamese twins that toured with Barnum and Bailey Circus in the late 1800s. They were brothers that were joined by skin right under the chest. Autopsy revealed their livers were also connected, making them hard to separate even if the technology was available at the time. They were identical, yet had very distinct and varying personalities. Chang was extroverted, happy most of the time, friendly, and a never consumed alcohol. Eng was very introverted, surly, had a dark outlook on life, sullen and withdrawn, and died because of complications due to his alcoholism. Identical twins, joined at the mid-section, yet different in every way.

D. Test resulting and decisions.

1. Good example is dwarfism. Certain types can be detected pre-natally. Questions that arise: Why are there dwarfs if the condition can be detected pre-natally? Comes down to measuring life on an economic scale. Person with this condition will have to deal with insurance costs, treatments, surgeries, special needs, etc. Looking on a web page devoted to dwarfs, most state this as "genocide" and say it lowers their self worth.

2. What is normal?

3. Whose decision is it to make?

E. Choosing "Superior" genes for unborn child.

1. Technology may make it available to pick and choose traits for an unborn child, i.e. better eyesight, increased memory, athleticism, better health, etc. Genes can be added to or manipulated to accomplish this fete. This is obviously advantageous to the child. This can be done to give a child a better chance to go farther in education, career, sports, etc.

2. Who should be granted this opportunity? The ones who can afford it, or everyone.

3. Should it and how should it be regulated?

4. What is "perfect"?

5. These altered children will eventually have children and these altered genes will be passed on with others. Will this increase evolution too quickly?

6. Are we helping or hurting mother nature?

F. Cloning for harvesting.

1. If human cloning is done, what will be done with the clones? Will it be okay to use cloned humans for parts, i.e. livers and kidneys, and hearts for transplant recipients. The genes may be the same as the person they came from, but the clone is not the same. Different personality, thoughts, opinions, etc. What regulations should be placed on this issue?

G. Other possible discussion topics: These are good for pro/con discussions.

1. Choosing the sex of a baby.

2. Growth hormone injections.

3. Insurance coverage.

4. Presymptomatic testing.
5. Gene therapy.
6. In-vitro fertilization.
7. Frozen embryo: personal use vs. research.

LABORATORY EXERCISES

DNA Isolation

(Mertens & Hammersmith, 1998. Exercise #15)

Materials needed

7 test tubes

1 stopper for a test tube

6 aluminum test tube caps or aluminum foil

1 250-ml beaker

1 1000-ml beaker

1 glass stirring rod

Water bath set at 100 C

Spectronic 20 or other spectrophotometer

6 spectrophotometer tubes

Ice and ice water

Pasteur pipettes and bulbs

Stock solution of DNA containing 1 mg DNA/ml

Supply of 5-ml pipettes

1 automatic pipetter of Dische reagent

1 centrifuge with 50-ml size rotor

Cheesecloth and 100-ml beaker

1 blender

1000 ml cold saline citrate solution

1000 ml cold 2.6 M NaCl solution

200 ml Dische reagent

1000 ml freezer cold 95% ethanol

Extraction and quantification of DNA

Beef or pork spleens from freshly slaughtered animals can be obtained from local slaughterhouses, returned to the lab in ice, and used to prepare a DNA solution as outlined in the following steps. Spleen is used because tissue of this organ contains a high percentage of nuclear material.

Chop up fresh spleen tissue into small cubes (1 cm3). Avoid using any fat or connective tissue which appears lighter in color. Use 10g spleen for every 100 ml DNA solution required. Approximately 50 ml of solution will be required for each group of four students.

For 100-ml DNA solution, place 100-ml cold citrate-saline buffer into a chilled blender. Drop pieces of spleen into the blender after it has been turned on. Blend for two to three minutes. After homogenizing the spleen, pass the homogenate through several layers of cheesecloth into a beaker to remove large clumps.

Distribute the homogenate between 50-ml centrifuge tubes (50-ml per tube) and balance the tubes before centrifuging. Mark the fluid level on each tube using a wax pencil.

Centrifuge for 15 minutes at 6000 rpm.

Discard supernatant in an approved site.

Pour 2.6 M NaCl solution into each tube to the level of the wax mark. Use a glass rod to vigorously shake and dissolve the pellet. Balance tubes.

Centrifuge for 15 minutes at 6000 rpm.

Pour the supernatant into a 250-ml beaker. Make an ice bath using the 1000-ml beaker and place the 250-ml beaker containing the DNA solution into this ice bath. Keep the DNA solution chilled.

Quickly dump 100 ml freezer cold 95% ethyl alcohol into the beaker containing the DNA solution, so that the alcohol forms a layer on top of the DNA solution. The DNA will precipitate at the interface so between the alcohol and the DNA solution.

Describe the appearance of the resulting DNA precipitate.

Using a glass stirring rod, stir the precipitate that forms at the interface of the DNA solution and the alcohol, winding the precipitate onto the rod. This threadlike precipitate is the DNA. Be sure to extract all the DNA that can be wound onto the rod.

What can be said of the physical nature of DNA?

Remove the stirring rod with the attached DNA from the beaker and gently drain and blot the alcohol from the DNA onto a paper towel. Removal of the alcohol will facilitate dissolving the DNA in water.

Transfer the DNA to a test tube containing 10 ml distilled water and gently remove the DNA from the rod by agitating the rod up and down. Try not to clump the DNA. Stopper the tube and shake vigorously until the DNA is in solution. Prolonged and vigorous shaking will be required to get all of the DNA into the solution.

Determine the concentrations of DNA in this solution, using the following serial dilution and quantification procedures. Label six test tubes from 1-6.

Place 2ml distilled water in tubes 2 through 6

Place 2 ml standard stock solution containing 1 mg DNA/ml in tubes 1 and 2. Mix the contents of each tube. Set aside tube

Transfer 2 ml from tube 2 to tube 3.

Mix the contents of tube 3 and transfer 2 ml from tube 3 to tube 4.

After mixing tube 4, discard 2 ml from tube 4. Note that tubes 1 through 5 all have 2 ml solution containing from 1 mg DNA/ml to no DNA (tube 5).

Place 2 ml of the beef spleen DNA solution from step 15 above to tube 6.

Mix 4 ml Dische reagent with the contents of each of the six tubes, and cap the tubes with aluminum caps or foil to prevent evaporation. Now, heat the tubes in boiling water for 10 minutes.

After cooling the tubes in ice water for a few minutes, pour the contents into spectrophotometer tubes bearing corresponding numbers. You are now ready to read absorbances on a spectrophotometer.

Use tube 5 (distilled water and Dische reagent) as a blank to zero the spectrophotometer, which is set at 500 nm.

Read each of the six tubes at 500 nm, recording each absorbance.

Plot the absorbances of tubes 1 through 4. Plot absorbances on the vertical axis and DNA concentrations on the horizontal.

Remembering that each 10 ml of the DNA solution contained DNA extracted from 1 g of spleen, calculate the percentage DNA in bovine spleen.

Mitosis

(Mertens & Hammersmith, 1998. Investigation #5)

Materials needed

Microscope

Microscope slides

Cover slips

Prepared slide of onion root tip mitosis

Prepared slide of human metaphase chromosomes with differentially stained chromatids

Acetocarmine stain

Hydrochloric acid

Watch glass

Dissecting needles

Scalpel or razor blades

Forceps

Alcohol lamp

Onion bulbs

Beakers, for onions to sprout

Colchicine solution

Absolute ethyl alcohol

70% ethyl alcohol

Glacial acetic acid

Chloroform

Slides of mitosis in whitefish blastula

Mitosis in onion root tip squashes

Onion bulbs will sprout roots if they are placed in water for several days. The bulbs should be placed in water about four days before the lab work is to be done. However, individual batches of onion bulbs will respond quite differently to conditions suitable for germination. Many onions obtained commercially have been treated to prevent sprouting. Consequently, they produce very few roots. Onion sets, or locally grown onions, will sprout more effectively.

Young, actively growing roots that are from 2.5 to 5 cm in length should be used for the study of mitosis. The cells near the tip of the root are actively dividing, thus causing growth.

Clip the terminal 1cm of the root tip from a growing onion bulb during the lab and use it immediately, or use a root tip harvested and preserved in Carnoy's solution (6 parts absolute ethyl alcohol:3 parts chloroform: 1 part glacial acetic acid) for 24 hours and then stored in 70% ethyl alcohol until the time of use.

To examine the mitotic process in the cells of the onion root tip, you must soften the root so that the cells can be separated and flattened, thus making it possible to see the chromosomes, nuclei, spindles, and other cell parts.

Place some 1M HCL in the watch glass. Be careful not to get the acid on your skin or clothing.

Into this acid place the terminal 3 or 4 mm of the 1 cm long onion root.

In a short time (a few minutes) the root tip will feel soft when touched with a dissecting needle.

Now, using forceps or a needle, pick up the softened root tip and transfer it to a drop of acetocarmine stain on a clean slide.

Using a razor blade or a sharp scalpel, chop the root tip into tiny pieces. Note: iron in the scalpel reacts with the acetocarmine stain to give a better staining effect.

Once this is complete, apply a clean cover slip to the slide and heat it gently over an open flame. Then invert the slide on a paper towel and push downward firmly, applying pressure with your thumb over the cover slip. This should flatten the cells and disperse them so they can be observed under the microscope.

Locate all the phases of mitosis.

What phase are most of the cells in?

The mitotic poison Colchicine can be used to reveal details of chromosome morphology, permit chromosome counts to be made, or induce polyploidy. Colchicine inhibits the formation of the mitotic spindle and thus stops the mitotic process at metaphase. Colchicine is an alkaloid compound derived form corn and other plant parts of the autumn crocus, *Colchicum autumnale*; it is used medicinally in low concentrations to treat gout.

Do you observe more cells in mitotic stages than you observed in the untreated root tip?

What mitotic stages are more prevalent?

Are any stages completely lacking?

Draw the stages of mitosis seen in your slides. If there are stages that are not evident in your sample, look at lab mates slides to find all stages and draw them.

Meiosis

(Mertens & Hammersmith, 1998. Investigation #6)

Materials needed:

Microscope

Slides of ascaris oogenesis

Slide of grasshopper spermatogenesis

Preserved and prestained grasshopper testis

Acetocarmine or aceto-orcein stain in dropping bottle

Microscope slides

Cover slips

Alcohol lamp

This lab will study meiosis at it occurs during the course of egg production in the roundworm. This parasite worm normally lives in the intestinal tract of horses.

Ascaris is widely used in the study of meiosis because its low chromosome number simplifies observation of individual chromosomes and their behavior. The species of ascaris we are using has two pairs (2N=4) of chromosomes in its diploid cells.

You will be given either one slide containing several (normally five) different sections of the uterus of the ascaris or four or five different slides, each containing a single section of ascaris uterus. Each section contains cells in different stages of maturation. The series presents all of the major events of oogenesis and early embryo development in ascaris.

If you have a slide with all the stages on it, focus on the top row using low power. Note the irregular shaped “eggs”, which are diploid cells that have not undergone meiosis.

Careful inspection of the cells will reveal they sometimes contain one or two small black spots. A perfect “egg” (primary oocyte) in this section of the uterus will contain two small masses of chromatin representing the synapsed (paired) homologous chromosomes.

Note the spermatozoa, which appear as very black, somewhat triangular objects. Many sperm cells will be seen among the oocytes. In other cases a sperm may appear inside the primary oocyte, because in ascaris the sperm penetrates the diploid cell prior to meiosis of the potential egg. The shape of the sperm will also be altered. An ascaris sperm lacks a flagellum, but used amoebid movement for locomotion.

Find an oocyte with two black masses near the shell. Locate an oocyte in which each of these masses appears to be made of four divisions (chromatids). Such a cell (primary oocyte) is in the tetrad stage. Each chromosome of a pair has become visibly duplicated, producing the tetrad. Some tetrads may be getting ready to divide. A spindle can also be seen, with the chromosomes located on it at metaphase I or anaphase I.

Try to locate tetrads dividing into two doublet structures (dyads). Many oocytes will have half of each of the two tetrads apparently attached to the shell as a single black mass. This is the first polar body. Such an oocyte is now called a secondary oocyte and each of the double chromatin masses remaining in its cytoplasm is called a dyad.

A perfect specimen of an egg will contain two nuclei: a female pronucleus, containing the chromatin of the two remaining chromosomes in a non condensed or

interphase-like stage. The male pronucleus, containing an interphase-like condition has the chromatin brought in by the sperm. In addition, a perfect egg will have its first polar body on the inside of the shell and its second polar body on the edge of the cytoplasm.

Spermatogenesis Slides

Place several drops of acetocarmine stain on a clean microscope slide.

Place a piece of preserved dyed grasshopper testis in the acetocarmine stain.

Crush the tissue with a flat end glass rod, breaking the tissue into small pieces.

Apply a coverslip and heat over an alcohol lamp, being carefule not to overheat. Invert the slide onto a piece of paper towel and press down firmly on the coverslip. This will disperse the cells.

Alternate Lab For Meiosis And Mitosis

Look at prepared slides of meiosis and mitosis. Draw stages and be able to identify all stages by sight or description.

Probability, Binomial Expansion, Chi-Square

(Elrod, White, and Elrod. 19)

Probability is the likelihood of an event based on the ratio between its occurrence and the average number of cases favorable to its occurrence taken over an indefinite series of such cases.

Toss a coin 50 times and record the results; heads v tails.

Calculate the expected number (e, heads 25 times, tails 25 times) and determine the deviation (o-e, o = observed). Sum the deviations.

If the deviations (o-e) obtained are small, this can be attributed to chance. If they are large, take this data and apply to Chi-Square.

Toss two coins together and record data. A) Heads/Heads B)Heads/Tails C)Tails/Heads D) Tails/Tails. Work out probability.

This is similar to the outcome of a monohybrid cross. When Aa produces gametes, the probability is that ½ of the gametes will contain the A allele and ½ will contain a. When an Aa female is crossed with an Aa male and progeny are produced, the probability is ¼ that an A egg and an A sperm will come together to produce AA offspring. Similarly, the probability is ½ for Aa and ¼ for aa progeny.

Calculate expanded binomial (a + b)n = 1. A = probability of first event, B = probability of second event, N = size of the group, number of traits, etc. Use sex of children for an example.

If we are looking for a family of four, what is the probability that two are girls and two are boys? In the expanded binomial, a4 + 4a3b + 6a2b2 + 4ab3 +b4; assume a= girls, b=boys. A2b2 describes two girls and two boys. Work out the equation:

6a2b2

6(1/2)2(1/2)2=

6/16 or 3/8

Give several different examples to work out expanded binomial.

Chi-Square equation; X2 = E(o-e)2/e. o = observed e = expected E = sum

Good Chi-Square example is to use PTC paper and tabulate numbers of taster and non-tasters in the class. To calculate expected, assume 80% are tasters. Calculate X2.

To interpret this value, determine the degrees of freedom by taking the number of cases and –1. (n-1)

Determine reject or accept by X2 value table. Give several problems to work out.

An in depth discussion of binomial expansion and ChiSquare would be helpful to fully understand why this is an important tool in research.

Drosophila Culture Lab

(Winchester & Wejksnora, 1996. Investigation #1)

Drosophila, the common fruit fly, was one of the fist animals to be used extensively in genetic breeding experiments and is still the most widely used for this purpose. It has short life cycle, large numbers of offspring, great variability in its inherited characteristics, and it easily raised in the lab. Its method of gene transmission and sex determination is the same as in most higher forms or animals including humans. They also contain large chromosomes in their salivary glands that are great for chromosome studies. These features all make the *Drosophila* almost ideal for use in beginning genetic lab to understand inheritance. This lab will teach how to culture these specimens for use in experiments.

Supplies:

Fly strains

Media in stopper bottles

Small, soft brush for handling flies

Felt-tip marker for labels

Anesthetizing chamber

Fly-Nap ® anesthetic

Anesthetic wand

Fly morgue with 1" of alcohol for disposing of flies

Preparation:

Drosophila can be raised on a variety of food; a bit of ripe banana in a jar will work. Removing the flies without the banana can be difficult. Most food media have some method of solidification to avoid this problem. The ingredients used in preparing the media will make a difference in the results obtained. Three can be noted: (1) The time of the generation cycle. This may vary from 9 days to 16 days at about 24 **C (75** F) (2) The number of flies obtained from a culture may show as much as fourfold variation. (3) The size of the flies obtained may show a twofold to threefold difference.

Selection of a food medium is very important, especially when the time for conducting the experiment is limited and a short generation cycle is essential. Addition of dry inactive yeast to whatever food is used will greatly improve the quality of the food. Flies thrive on yeast in fermenting fruit in their native state, and the extra yeast will add to its nutrients.

Instant Culture Media

Several media can be considered: Formula 4-24 ® is being used in this description.

To prepare culture, obtain a plastic culture vial. Place a capful of culture media in the vial. Pour one measuring cap of water into the vial. Lightly swirl the vial so the mixture becomes rehydrated. Several grains of yeast must be added to the vial since the yeast serves as a food source. The medium will be quite fluid at first, but within a few

minutes, it will become more solidified and will be ready to use. A foam stopper can be used to close the opening of the vial, yet allowing for gas exchange.

Storing:

This media can be stored in the refrigerator for one to two weeks if kept in plastic bags or other airtight containers to prevent dehydration. To prevent molding, sprinkle a few grains of fresh, active yeast granules on the surface of the food. This yeast will make a growth that will not leave room for mold spores to germinate and start mold growth. It is a good idea to allow the food to warm to room temperature before introducing it to the flies.

Anesthetizing *Drosophila*

Use product such as FlyNap ® to anesthetize the flies without killing them due to overexposure to ether. Flies will usually be anesthetized for thirty to forty-five minutes.

Place the anesthetizing wand into the vial of flies. If the vial is empty, it should take two to three minutes. If the vial has medium, four to five.

Distinguishing Sex

The most noticeable sex difference is in the dorsal surface pigmentation of the posterior abdomen. The male has heavy pigmentation on this part with two bands. The female has five bands of pigmentation along the entire abdomen.

In young flies, sex determination is much harder. The posterior tip of the female is somewhat pointed. In the male it is more rounded.

The males will also have a pair of dark, bristle-like sex combs, one on each fourth joint of each leg. Males are also notably smaller in most cases.

When flipped on their back, the male genital area is surrounded by by heavy dark bristles.

Drosophila Life Cycle

Flies are usually ready for mating within ten hours after emerging from the pupa case. The courtship entails the male circling the female while vibrating his wings. The female will spread her wings laterally in response.

The sperm is received in receptacles within the female and will be used to fertilize eggs laid by the female for her entire life. Hence, it is not possible to cross the same female to different males at different times to achieve different results. Sperm from the first mating will still be present.

Eggs are usually fertilized at about the time of laying, and early embryonic development takes place within the egg case. Eggs are usually present within one day of the flies being placed in the vial. They are about 0.5 mm long and have two filaments on the anterior end to prevent the eggs from sinking into the food.

Within two days after the eggs have been laid, very small larvae will hatch. This represents the first instar. It will eat constantly and spend double its size about every 24 hours.

Within another two days it will molt and form the second instar. This will eat and molt again to form the third instar, which is up to 5 mm in length.

Pupation comes at the sixth or seventh day. The third instar adhere to a dry surface to dry out. They develop a brown pigmentation. The fly is now a pupa case, and it is within this stage the fly changes from larval to adult.

Within three days the adult emerges from the anterior end of the case. The newly emerged fly will be very long, relatively unpigmented, and with folded wings. It will darken rapidly and the wings will unfold while the body becomes more compact.

Mutant Genes of *Drosophila*

As stated earlier, *Drosophila* are commonly studied in breeding experiments due to their large salivary glands that are ideal for chromosome study. In doing these breeding experiments, mutants may and will arise. This is a tabulation of some of the most easily recognizable mutant genes. All are recessive to the wild type unless indicated otherwise. The letter gene symbol is given after the name of each mutant.

Chromosome I (the X-chromosome)

Apricot eye (w-a)- apricot colored eye, an allele of white, has no dark spot. The color varies slightly in homozygous females as compared to hemizygous males.

Barr eye (B)- eye narrowed vertically to form a bar, intermediate in effect. Vary narrow bar in homozygous females and hemizygous males, but a wider bar in heterozygous females.

Crossveinless wings (cv)- cross veins of wings missing, longitudinal wings normal.

Eosin eye (w-e)- bright orange-red eye color, an allele of the gene for white, no dark spot.

Forked Bristles (f)-bristles on thorax are split at the ends and form a fork as they curl away from the center. The curled ends often break off so that only one curled end is present, or none at all, in which case the bristles just seem short and stubby. Body hairs are also affected.

Miniature wings (m)-wings normal shape, but reduced in size, extend only about to the posterior tip of the abdomen; wild type long wings extend well beyond abdomen.

Vermillion eye (v)- eye a bright scarlet red, dulling somewhat with age; not an allele of white; has no dark spot. When both vermillion and brown are expressed, the eye is white or sometimes a very pale pink. Color is indistiguishable by sight from cinnabar and scarlet since all represent absence of brown pigment, but for different reasons.

White eye (w)-white eye.

Yellow body (y)-body a distinct yellow color in contrast to the gray body of the wild type; bristles are brown with yellow tip, wild type are black.

Chromosome II

Black body (b)-body has heavier pigmentation than the wild type gray body, mutant has a shield shaped figure in the center of dorsal surface of the thorax. This feature can be distinguished even in newly emerged flies when the overall pigmentation is rather light.

Brown eye (bw)- eyes a light brown on emergence, darkens with age, no black spot. Also causes testes to be colorless and transparent so can be recognized in larva as well as adults. White or very pale pink eyes result when both brown and vermillion are expressed.

Cinnabar eye (cn)-eye very bright scarlet red on emergence, dulling somewhat with age, no dark spot, same color as vermillion and scarlet. Results in a white eye when homozygous along with brown.

Curly wing (Cy) – wings curve upwards, dominant gene that is lethal when homozygous.

Curved wing (c) –wings curve downward and are held somewhat in a spread position when the fly is at rest.

Dumpy wing (dp) –wings are shortened to about the length of the body, but differ from miniature in that the ends are squared off and have an indentation in them. They are broader at the posterior end than miniature.

Lobe eye (L) – eye is narrowed with an indentation at its anterior edge. Dominant gene that is lethal when homozygous.

Star eye (S)- facets of the eye are roughly and easily distinguished from the smooth eye of the wild type. Dominant gene.

Vestigial wing (vg)- wings reduced to mere stumps that protrude laterally from the body. When raised at unduly high temperatures (26.5 C or 80 F and above) the wings may expand into a somewhat larger size, but will be deformed.

Chromosome III

Dichaete wings (D)- dominant gene that causes wings to be held out to the side rather than parallel to the body. Also shortens the bristles somewhat. Lethal when homozygous.

Ebony body (e)- much heavier pigment deposits on body, shield-shaped figure of even heavier deposits on dorsal surface of thorax. Homozygous flies are darker than those homozygous for the gene for black (b/b). Pigment becomes progressively darker with age, but shield on thorax makes distinction of newly emerged flies clear cut.

Glass eye (gl)- facets fused to give smooth, glossy appearance.

Scarlet eye (st)- bright, scarlet-red, dulls with age; gives white when expressed along with brown. No dark spot on eye, identical in color to vermillion and cinnabar.

Sepia eye (se)- transparent brownish red, darkens with age, no black spot.

Chromosome IV

Eyeless (ey)- eyes reduced in size, but show great variation in expressivity. A few have no eye at all, while in others it may be only slightly smaller than the wild type eye size. Also varies in the eyes of the same fly.

Stubby bristles (Sb)- the bristles are short and stubby. Dominant gene, lethal when homozygous.

Sex Chromosomes and Gene Transmission

(Mertens & Hammersmith, 1998. Investigation #9)

Procedure:

For this experiment, you may use any one of many X-linked mutants in Drosophila. The following discussion is based on the assumption that you will use the X-linked white eye (w) mutant.

Cross – some students will mate a white eyed virgin female fly (XwXw) to a wild type male (X+Y). Produce F1 and F2 generations and record data. Other students will mate a wild type virgin female (X+X+) to a white eyed male (XwY) to produce F1 and F2 generations and record data. The two crosses will be compared.

Cross _____ female x _____male

Date P1's mated _____

Date P1's removed _____

Date F1's first appeared _____

Phenotype of F1 males _____

Phenotype of F1 females _____

Date F1 male and female placed in fresh bottle _____

Date F1 flies removed _____

Date F2 flies appear _____

F2 data:

Males:		Females:		
Phenotype	Number	Phenotype	Number	Total
a.				
b.				
c.				
d.				
Totals: _____		_____		_____

Explain how the trait you have been studying would be inherited in a reciprocal cross if it were not controlled by a gene that is X-linked.

Comparison data:

Cross (A)		Reciprocal Cross (B)	
Phenotype	Genotype	Phenotype	Genotype
P1 female			
P1 male			
F1 female			
F1 male			
F2 female			
F2 male			

Mating involving an attached X-female drosophila:

In the mitotic and meiotic processes, occasional errors will occur when chromosomes fail to separate at anaphase and the two members of a pair or the daughter chromatids of a single chromosome go to the same spindle pole. Such failures of chromosomes to separate is known as nondisjunction. Special drosophila stocks exists in which compulsory nondisjunction of the X chromosome occurs because the two X chromosomes of the drosophila female are physically joined together at their centromere ends. Such an "attached X female" may have a Y chromosome as well and thus have the chromosomal condition XXY. Such a female is viable and fertile. When meiosis occurs, she produces two kinds of eggs, those bearing two (attached) X chromosomes and those bearing Y chromosomes.

When such a female is mated to a normal XY male, what chromosomal constitutions may be expected among the resulting zygotes?

Would you expect all of these zygotes to be viable and to result in the production of adult flies?

The zygotes having what chromosomal constitutions might be suspected of not being viable?

What is unusual about the way in which the male offspring of such a cross (XXY x XY) come into being?

Mate a virgin wild type female fly having attached X chromosomes to a male that expresses an X-linked mutant gene such as that for white eyes (w). Produce F1 and F2 generations and record data.

Give the genotypes of the P1, F1 and F2 flies from the cross you performed. Show X-linked genes as superscripts on the X chromosomes.

P1 female _____

P1 male _____

F1 female _____

F1 male _____

F2 female _____

F2 male _____

On the basis of the results you obtained in this experiment, can you now say with certainty which zygotes resulting from a cross of XXY to XY are viable and which are inviable?

What would happen in the next generation if you were to allow the F2 males and females from this cross to mate inter se (among themselves)? What genotypes and phenotypes might be expected in the next generation?

Based on the information obtained in this experiment, what is the role of the Y chromosome in sex determination?

Mutations

(Winchester & Wejksnora, 1996. Investigation #9)

Procedure:

Collect virgin females showing the mutant and mate them to males of a special stock of flies that is designed to determine the linkage group crosses involving only two generations. Male stock can be curly, plum, dichaete, or stubble. All of these are

dominant. Cy and Pm are on chromosome II, the others are on chromosome III. This is a balanced lethal stock.

When the F1 emerge, examine them for the appearance of your unknown mutant characteristic. If the male shows the mutant and the female does not, you know that your unknown gene is X-linked, one chromosome I, and you need not go further.

If your mutant does not appear at all, you will know that it is recessive and located on one of the three autosomes. To determine which one, obtain several virgins females showing the mutant and cross with a male showing any two of the dominant genes.

From this crossing you should determine the linkage group of your mutant.

Questions:

Give a complete description of the appearance of the unknown mutant that has been issued to you.

Give the symbols and the phenotypic effects of the dominant marker genes used in the balanced lethal stock (Chromosome II; Chromosome III).

Date mutant female were crossed with balance lethals?

Date parents removed from vial?

Does the mutant characteristic show in the F1?

If so, does it show in males only?

If the answer to the last two questions is “yes”, do not carry your cross any further. Skip to the next set of questions. If the answer to either question is “no”, then continue on.

What combination of dominant characteristics do you choose in the male selected to continue the experiment? Why?

Date mutant virgins mated with selected male.

Date parents removed

"Yes" questions:

According to the results obtained in the first and second crosses, upon what chromosome is your mutation located? Give reasons.

Why are sex linked genes easier to detect than autosomal genes in studies to determine linkage groups?

Give the symbol, phenotypic effect, and location of the dominant markers used to locate your mutant gene on its chromosome

Date male mutants crossed with virgins carrying dominant markers.

Date parents removed.

Tabulate the offspring, indicating which are crossovers and the percentage of crossovers between each marker and the mutant.

Linkage and Crossing Over

(Mertens & Hammersmith, 1998. Investigation #12)

Material needed:

Drosophila stocks – wild type; mutant stock with yellow body, forked bristles, miniature wings (yfm); other stock or stocks selected by instructor.

Linkage may be measured for either X-linked or autosomal genes. In the former case, if a multiple-mutant female is mated with a wild type male to produce and F1, one

can subsequently produce and F2 in which crossover percentages can be readily measured. By contrast, if the genes are autosomal, one must test cross an F1 female to determine crossover percentages conveniently.

Now mate the fly stocks provided by the instructor. For example, you might be asked to mate a virgin yfm female with a wild type male. Produce the F1 flies and determine whether the genes involved are autosomal or X-linked.

If the genes are autosomal, test cross virgin F1 females with multiple mutant males. Record all data relative to this cross and the test cross. Also perform the reciprocal cross. Mate an F1 male with a virgin multiple mutant female. Record all data from this cross. If the genes are X-linked, mate an F1 female with an F1 male, produce an F2 generation and record all data.

Calculate the Chi-Square value for each of the three traits by themselves. Also calculate the X2 on the basis of the hypothesis that the three traits are independently assorted.

Sex Chromatin in Human Cells

(Mertens & Hammersmith, 1998. Investigation #10)

Materials needed:

Microscope equipped with oil-immersion objective

Tongue depressor

1% aqueous solution of thionin stain

Ethyl alcohol in the following concentrations: 50% 70% 95% 100%

Xylene

5 or 6 normal hydrochloric acid in dropping bottle

Balsam mounting medium

Microscope slides

Cover glasses

Coplin jars or dishes

Forceps for handling clean slides

Obtaining the smear:

Clean a slide thoroughly in detergent, rinse in distilled water then rinse again in 70% ethyl alcohol. Flame dry the slide. The slide needs to be uncontaminated to be used.

In order not to contaminate the epithelial cells, students need to thoroughly rinse their mouths with tap water several times to remove bacteria.

Prepare the smear by GENTLY scraping the lining of the cheek with a tongue depressor and smearing the material onto a clean slide. Air dry the slide for thirty seconds.

Place the slide in 95% ethyl alcohol for 2 minutes, then in 70% ethyl alcohol for 2 minutes, then in 50% ethyl alcohol for 2 minutes, then finally in distilled water for 2 minutes.

Upon removal of the slide from the distilled water, place several drops of 5 or 6N HCL acid on it for 5 seconds.

Transfer the slide to distilled water for 10 – 15 seconds to remove the HCL acid.

Stain the slide in 1% aqueous thionin stain for 10 – 15 minutes. Rinse the slide in distilled water.

Dehydrate slide according to the following schedule: 50% ethyl alcohol for 30 seconds, 70% ethyl alcohol for 30 seconds, 95% ethyl alcohol for 30 seconds, and 100% ethyl alcohol for 30 seconds.

Transfer the slide to xylene for clearing for 1 minute. After the xylene, immediately place a drop of balsam mounting medium over the cells and apply a clean cover slip. Avoid trapping bubbles.

Examination of smears prepared this way should reveal cells with virtually colorless cytoplasm and a pale blue nucleus. The sex chromatin bodies should be stained a dark blue-black. Record data on how many sex chromatin bodies were found in class. How many in a female cell? Male cell? Where they're more than one in a female? As the number of sex chromatin bodies increases, what would the total number of chromosomes be?

Human Genetics

(Winchester &Wejksnora, 1996: Investigation #11)

Procedure:

Characteristics being studied:

Attached earlobe	recessive
Widow's peak	dominant
Tongue rolling	dominant
Bent little finger	dominant
Hitchhiker's thumb	recessive
Long pulmar muscle	recessive

Pigmented iris recessive

PTC taster dominant

Mid-digital hair dominant

Make a chart tabulating phenotypical characteristics of the list above. Make room for individual, class total, and genotype.

Categorize the chart further by dividing characteristics by male and female.

Call out each characteristic and have students stand that have them.

How many characteristics must be considered before you stand out as an individual?

What is the average number for the class?

What proportion of the above characteristics would you expect to find the same in identical twins? Explain.

Analysis of Inherited Characteristics.

List two characteristics that have been chosen and determine the chance of each showing in any person picked at random.

Characteristic #1. _____

Number of persons in the class who show it. _____

Total number of persons in class. _____

Percentage showing it. _____

Characteristic #2 _____

Number of persons in the class who show it _____

Percentage showing it _____

Consider the percentage showing a characteristic as the chance of any one person showing it. Now calculate the chance of any one person showing both characteristics._____

According to the figures obtained above, how many in the class would be expected to show both of these characteristics? _____

How many in the class actually show these two characteristics? _____

What is the deviation? (O-E) _____

Does the deviation appear to be significant in light of the number of persons involved in your studies? Use Chi-square to determine._____

If you show a recessive trait and you marry a person who does not show it, what are the chances that it will appear in your first child? First calculate the probability of having a child that is recessive if the person you marry is homozygous dominant. Then calculate the probability of having a recessive child if you marry someone whom is heterozygous. The two calculations are added to give your answer.

Pedigree Analysis

(Elrod, White, & Elrod, 19)

This lab should be used to reiterate the lecture on pedigree and genetic counseling.

Distribute a legend of pedigree symbols to be discussed in lab.

To illustrate the use of a pedigree, obtain an example with four generations.

An example to show inheritance can be X-linked hemophilia. Mark affected II 1, II 2, III 3, III4, III 6 (males of course) or any other trace that fits.

Determine probabilities for affected individuals and carriers.

Use this same technique for tracing other examples, i.e. albinism.

Diagram your family pedigree indicating those that express a trait you have chosen to analyze. (For discretion, have students do a make believe trait to follow if needed.)

According to the pedigree, what seems the most likely method on inheritance of the trait being studied? _____

Is there an alternative possibility? What? _____

How far back is this trait visible? What is the probability of future affected or carrier?

If student is not married, have them put in a spouse to determine probabilities.

Or, if for discretion reasons, it is decided not to do own family pedigree, give an example of a family throughout four to six generation and have the students fill in affected, carriers, and figure probability.

Karyotyping

(Elrod, White, and Elrod, 19)

This lab should be used to reiterate the lecture on human karyotyping and chromosome etiology.

Standard system of chromosome nomenclature is referred to as the Denver System.

Group A – consists of the first three pairs. They are distinguished from one another because of length and centromere position. A secondary constriction is sometimes seen in the proximal portion of the long arm on chromosome number 1.

Group B – consists of chromosomes 4 and 5. The are shorter than group A and have subterminal centromeres.

Group C – consists of chromosomes 6 through 12 and the X chromosome. Chromosomes 6, 7, 8, 11, and the X chromosome are metacentric. Chromosomes 9, 10, and 12 are submetacentric.

Group D – consists of three chromosomes pairs that are difficult to distinguish. They are acrocentric, with small satellites that are not always visible.

Group E – consists of chromosomes 16, 17, and 18. Chromosome 16 sometimes has a secondary constriction in the proximal portion of the long arm.

Group F – consists of chromosomes 19, and 20 which are metacentric.

Group G – consists of chromosomes 21, 22, and the Y chromosome. Chromosomes 21 and 22 have satellites, and the Y chromosome has long arms that lie close to each other.

Using the above guidelines, prepare a G-banded karyotype from a photographic print. Make sure to line chromosome along number line. Make sure to determine any chromosomal anomaly that the karyotype may represent.

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